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CASWELL FILE

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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PC
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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Prothiophos, Use on Sand Pears, PP#5E3182.
Caswell #714H

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

FROM: Brian Dementi, Ph.D. B. Dementi
Review Section 1
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: George LaRocca, PM #15
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

THRU: R. B. Jaeger, Section Head 2/11/86
Review Section 1
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Recommendation:

Data base does not support the proposed tolerance on sand pears.

An overriding concern with respect to all studies is the lack of information as to the purity and composition (particularly with respect to dioxin contamination) of the test material(s). Accordingly, all studies herein reviewed are to be considered invalid pending receipt of analytical information. Upon receipt of such information, and contingent upon the findings, the core classifications of the various studies can be upgraded to those indicated in each case below.

Findings: Data submitted with this petition are summarized below.

1. Title: Study for Acute Oral and Cutaneous Toxicity and also Acute Inhalation Toxicity and Skin and Mucosa Toleration.

Core Classification: Invalid

2. Title: The Studies of Toxic Effect by Simultaneous Administration with Tokuthion and Several Pesticides.

Core Classification: Supplementary
Test Animal: Mouse, male
Tokuthion (Prothiophos) LD_{50} = 560 mg/kg

3. Title: NTN 8629 Effect on Cholinesterases.

Core Classification: Minimum
Test Animal: Rat, male
Cholinesterase:

	LEL, mg/kg	NOEL, mg/kg
Brain	97	49
Plasma	9.7	4.9
Erythrocyte	4.9	Undetermined (< 4.9)

4. Title: Subacute Toxicological Studies on Rat (3-Month Feeding Experiment).

Core Classification: Invalid

5. Title: Subacute Toxicological Studies on Mouse (3-Month Feeding Experiment).

Core Classification: Supplementary
Test Animal: Mouse, male and female
Cholinesterase:

	LEL, ppm	NOEL, ppm
Brain	125 (M, F)*	25 (M, F)
Plasma	5 (M, F)	1 (M, F)
Erythrocyte	1 (F), 25 (M)	Undetermined (< 1) (F), 5 (M)

*For purposes of comparison, 125 ppm prothiophos in the diet corresponded to averages of 31.3 and 46.1 mg a.i./kg/day for male and female animals, respectively.

6. Title: 6-Month Feeding with 1-Month Recovery on Rats.

Core Classification: Invalid

7. Title: Tumorigenicity Study in the Rat.

Core Classification: Invalid

8. Title: Tumorigenicity Study in the Rat II.

   Core Classification: Invalid

9. Title: Tumorigenicity Study in the Mouse.

   Core Classification: Invalid

10. Title: Evaluation of the Teratogenic Effect of NTN 8629 in the Rabbit.

    Core Classification: Supplementary

    Increased Resorptions and Fetal Deaths (Developmental Toxicity)

LEL, mg/kg	NOEL, mg/kg
50	15

    Study is Inconclusive with Respect to Teratogenic Potential of Test Substance.

11. Title: Administration to Rat During Organogenesis Period.

    Core Classification: Supplementary
    Dilated Renal Pelvis and Hydroureter (Developmental Toxicity)

LEL, mg/kg	NOEL, mg/kg
4	0.8

12. Title: Insecticide NTN 8629 - Administration to Pregnant Rabbits During Organogenesis Period.

    Core Classification: Invalid

13. Title: Evaluation of the Reproductive and Teratogenic Effects of NTN 8629 in the Rat

    Core Classification: Invalid

14. Title: Administration to Rat Before and During the Early Stage of Pregnancy.

    Core Classification: Supplementary
    Test Animal: Rat, male and female
    Reduced Thymus Gland Weight in Male Rats

LEL, mg/kg	NOEL, mg/kg
2	Undetermined

15. Title: Administration to Female Rats During Peri- and Postnatal Periods.

Core Classification: Invalid

16. Title: NTN 8629 - Multigeneration Study in Rats.

Core Classification: Supplementary

Increased Mortality among F_3B Generation Pups and Failure of F_2B Animals to Produce an F_3C Generation.

LEL, ppm	NOEL, ppm
5	Undetermined (< 5)

17. Title: A Multigeneration Study of NTN 8629 in Rats.

Core Classification: Supplementary

Increased F3 Generation Body Weights, Male

LEL, ppm	NOEL, ppm
3	Undetermined (< 3)

18. Title: Mutagenicity Test on Bacterial Systems with Prothiophos, January 1977.

Core Classification: Invalid

19. Title: Mutagenicity Test on Bacterial Systems With Prothiophos, September 1978.

Core Classification: Invalid

20. Title: Tokuthion. Antidotal Test on Rats and Mice.

Core Classification: Supplementary
Test Animals: Rats (male), Mice (male)
Mortality with Prothiophos Alone:

	LEL, mg/kg	NOEL, mg/kg
Rats	1500	1000
Mice	1000	500

Certain substances tested as antidotes, including atropine, 2-PAM and toxogonin (BH6), increased the prothiophos LD_{50} values and hence were shown to have antidotal activity.

21. Title: Study to Evaluate Effect of Antidotes After Oral Administration of NTN 8629.

Core Classification: Supplementary
Test Animal: Rat, female
Toxic Symptoms, Unspecified

LEL, mg/kg	NOEL, mg/kg
750	Undetermined

Data Deficiencies/Requirements

The following studies are required in accordance with §158.135 to support the proposed tolerance:

Acute Oral Toxicity - Rat
Acute Dermal Toxicity
Acute Inhalation Toxicity - Rat
Primary Eye Irritation - Rabbit
Primary Dermal Irritation
Dermal Sensitization - Guinea Pig
*Acute Delayed Neurotoxicity - Hen
90-Day Feeding Studies - Rodent, Nonrodent
*1-year Nonrodent (dog) Feeding Study
Oncogenicity Study - 2 Species, Rat and Mouse Preferred
Teratogenicity - 2 Species
Reproduction, 2-generation
Gene Mutation
Structural Chromosomal Aberration
*Other Genotoxic Effects

*Data gaps previously identified as missing. See memorandum of B. Dementi to W. L. Burnham (April 26, 1985) which identifies these as missing, and incorporates a November 1984 mini-review by Dr. Reto Engler, who identified the starred items listed above as being required.

Note: A 90-day neurotoxicity study may be required, dependent upon the results of the acute delayed neurotoxicity study. Subchronic 90-day feeding studies in the rodent and nonrodent may not be necessary if adequate chronic feeding studies in the rodent and nonrodent are provided.

Residue Chemistry Branch Deferrals

Re: 5/16/85 RCB memo "PP #5E3182, Prothiophos (Tokuthion®) in/on Japanese Sand Pears. Evaluation of Analytical Method and Residue Data (Accession No. 073099) (RCB #389)"; V.F. Boyd, chemist.

A. With regard to deferral 1)A) under Conclusions, Toxicology Branch has not adequately addressed the toxicity of prothiophos and, therefore, it is inappropriate at this point to comment on the toxicological significance of the oxon and/or other possible residues in RACs.

B. With respect to the RCB deferral under Appendix-CBI, Toxicology Branch considers it inappropriate to comment on a "what if" type of conclusion. Toxicology Branch concurs with RCB that the registrant must identify the manufacturing process starting materials, unknowns, etc. in order for any informed comment to be made, or estimate of relative importance or hazard for the unknowns.

When the information/data requested by TB and RCB are submitted and reviewed, TB will be in a better position to give informed comment on these RCB deferrals.

Study: NTN 8629 - Study for Acute Oral and Cutaneous Toxicity and also Acute Inhalation Toxicity and Skin and Mucosa Toleration.

Laboratory: Bayer AG, Institute of Toxicology
Wupperal-Elberfeld

Study Number and Date: Tab 4, January 22, 1975

Accession Number: 073098

Material Tested: NTN 8629 (purity unknown except that it is "technical" grade)

Animals: Wistar II Rats
New Zealand Rabbits

Acute Oral, Dermal, and Inhalation Studies

I. Acute Oral Toxicity

The acute oral toxicity was evaluated in rats by the oral administration of NTN 8629 to groups of 15 males and 15 females per dose. The compound was emulsified in a mixture of water and cremophor and was administered by stomach tube. The animals were then observed for toxic effects for 14 days. Male rats received dosages ranging from 100 to 1500 mg/kg spanning eight different dosage levels. Females were administered seven different dosage levels being employed over the same dosage range. For male rats, the LD_{50} was 966 mg/kg, the highest dose without findings was 100 mg/kg, and the lowest lethal dose was 750 mg/kg. For female rats the LD_{50} was 991 mg/kg, the highest dose without findings was 100 mg/kg and the lowest lethal dose was 750 mg/kg.

Toxic effects occurred in 4 to 24 hours following administration and were characterized by dyspnea, reduced general well being, and signs of inhibited cholinesterase activity. Onset of death occurred within 1 to 8 days of administration.

This acute study dose provides an estimate of LD_{50}, lowest lethal dose, and no-effect level as determined using an appropriate number of male and female Wistar II Rats (weight 165 to 230 gms).

The following comments address deviations from the Guidelines (Subpart F):

1. Individual rat observations are not provided.

2. No indication is given as to the volume of liquid that was administered during dosing. This figure according to guidelines should not exceed 1 to 2 ml/100 gms body weight.

3. There is no indication animals were fasted prior to substance administration.

4. As to observations on animals following dosing, there are no recorded cageside observations of the type mentioned under 6 (iii) of the guidelines.

5. Periodic weighings not recorded.

6. Identity of test substance not adequately defined.

Core Classification: Invalid

II. Acute Dermal Toxicity

NTN 8629 in concentrated form was applied at 1 ml/kg body weight to the shorn dorsal skin of five male and five female rats. The animals abdomen and back regions were then covered with aluminum foil and wrapped with adhesive. This covering was removed after 24 hours and the skin cleaned with soap and water. Within 24 hours of application of NTN 8629, the animals' well being was compromised, lasting 6 to 7 days. There were no deaths. The LD_{50} for male and female rats was reported as > 1.0 ml NTN 8629/kg body weight.

No further data were provided from the study. Substantial deviations exist with respect to the guidelines. These include:

1. Composition of NTN 8629 not provided.

2. Only one dose level was employed, hence, no dose response data obtained. This is all right provided it meets the requirements for single dose LD_{50} study employing 2000 mg/kg.

3. Overall details of procedure lacking.

4. Lack of cageside clinical observations as described under 8(iii).

Core Classification: Invalid

III. Inhalation Toxicity

Groups of 10 male and 10 female rats were exposed to the test compound in an "inhalation apparatus" for 1 hour and for 4 hours. Following exposure, the animals were observed for toxic effects over a period of 14 days. The tests were performed in an inhalation apparatus where NTN 8629 was sprayed in admixture with ethanol and polyethylene glycol 400 (1:1). The NTN 8629 content in the inhaled air was determined by gas chromatography and thermionic nitrogen detector.

Results were reported for males and females taken together as follows:

LC_{50} at 1 hr exposure > 242 mg/m^3 air
at 4 hr exposure > 271 mg/m^3 air

Toxic symptoms were manifest only in the 4-hr exposure group at the highest dose of 271 mg/m^3. These symptoms were described simply as reduced well being for a few hours. There were no mortalities at any concentration employed.

This study was presented in very abbreviated form. In addition to citing the above findings, only one table of data was presented, providing toxicological responses to only a few dosage levels for periods of exposure of one or four hours each. Deviations from guidelines include the following:

1. Composition of test material (i.e., analytical purity) not provided.

2. Doses were not spaced in such a manner as to provide a dose response. All responses were noted only at the highest dose.

3. Adequate full description of the exposure chamber employed was not provided. What exactly was the "inhalation apparatus" employed?

4. Temperature and relative humidity in test system were not provided.

5. Actual concentrations of the test substance in the atmosphere at the animal breathing zone is not reported.

6. Aerosol dynamics not described.

7. Comments on food and water disposition not provided.

8. Cageside observations, such as described at (9)(ii), not provided.

9. No animal weight data during the study provided.

10. Core Classification: Invalid

IV. Skin Function Tests

A. Test on Rabbits' Skin. A one paragraph (three sentence) description is provided for a study involving the application of 0.5 ml NTN 8629 to the inside region of the ear of the rabbit. Slight redness lasting 3 to 4 days was reported only after applying the material for as long as 24 hours. The comments constitute the extent of the findings.

B. Test on Rabbits' Eye Mucosa. Application of 0.1 ml NTN 8629 to the conjunctival sac of the right eye of two rabbits resulted in redness after 1 hour of exposure. This redness disappeared after 2 to 3 days. No alterations of the cornea were observed. These comments constitute the essence of the report.

Core Classification for both studies is Invaild - Summary only; no description of test substance.

Core rating cannot be upgraded.

Study: The Studies of Toxic Effect by Simultaneous Administration With Tokuthion and Several Pesticides

Laboratory: Nitokuno Agricultural Chemicals Institute, Laboratory of Toxicology

Study Number and Date: Report No. 50-3; Tab 7: December 22, 1976

Accession Number: 073098

MRID:

Material Tested: Tokuthion plus other pesticides.

Animals: Male mice (ddy-strain)

This study was undertaken to determine the acute toxicity (LD_{50}) of tokuthion and four other pesticides, and further, to assess the LD_{50} of equipotent mixtures of tokuthion and each of the other pesticides.

This is a short study which can be adequately described as follows:

Initially, the LD_{50} for each of the below-named five compounds was determined singly. The number of animals used per determination was not stated. The compounds diluted in water were administered by stomach tube to mice employing a constant volume of administration of 0.1 ml/10 gms body weight. The LD_{50} values in mg/kg for each compound thus determined were reported as follows: tokuthion, 560; malathion, 1100; dipterex, 500; hinosan, 120; EPN, 27. The study notes that the typical symptoms of cholinesterase inhibition were observed, but no measurements of cholinesterase activity were submitted.

Mixtures of tokuthion and each of the other pesticides were prepared by combining amounts of each which were of equivalent potency (LD_{50}) as determined above. Each of the four mixtures so prepared were similarly administered to mice over the dosage range necessary to assess the LD_{50} for the mixture. Obviously, if the toxicities of tokuthion and any of the other pesticides in question are additive, the LD_{50} for that mixture would be expected, i.e., equivalent to the mean of the LD_{50} values of tokuthion and the other pesticide as determined individually.

The actual expected and observed LD_{50} values and their ratios as determined for the four mixtures in question are (table 7, p. 6):

Combination	Expected LD_{50}	Observed LD_{50}	Ratio (E/O)
Tokuthion & Malathion	830 mg ai/kg	185 mg ai/kg	4.49
Tokuthion & Dipterex	530 mg ai/kg	1030 mg ai/kg	0.51
Tokuthion & Hinosan	340 mg ai/kg	285 mg ai/kg	1.19
Tokuthion & EPN	294 mg ai/kg	285 mg ai/kg	1.03

Conclusion:

The results show that the effects of tokuthion and malathion are synergistic and that tokuthion and dipterex could be described as antagonistic. Hinosan and EPN appear to be essentially additive with tokuthion.

Little else can be derived from the limited study, except that it provides an additional LD_{50} value for tokuthion for comparison with that of other studies.

Core Rating:

Supplementary

Study: NTN 8629 Effect on Cholinesterases

Laboratory: Bayer AG, Institute Fur Toxikologie, Wuppertal-Elberfeld

Study Number and Date: Volume 2, Tab 27; October 23, 1975

Accession Number: 073093

Material Tested: NTN 8629 (93.2% purity)

Animals: Wistar II Albino Rats

NTN 8629, emulsified in a mixture of cremophor and water, was administered once by gavage to male rats in groups of 5 rats per dose. The compound was administered in a constant volume of 10 ml/rat. Dosages employed were 4.9, 9.7, 24, 49, 97, 291, 730, and 880 mg/kg. Samples of blood were withdrawn from rats for serum and erythrocyte cholinesterase determinations at intervals of 2 hours, 5 hours, 24 hours, 2 days, 7 days, and 14 days following NTN 8629 administration.

At the lowest dose, 4.9 mg/kg, erythrocyte cholinesterase was inhibited by 2 hours post-administration. The inhibition increased with increasing dose at the 2-hour time interval and also increased with increasing time, reaching a maximum inhibition at 24 to 48 hours postadministration. Measurable inhibition of this enzyme at the highest two doses was evident 14 days post-administration. Plasma cholinesterase inhibition at the lowest dose was not evident at any time point. At the 9.7 mg/kg dose slight inhibition of plasma cholinesterase was first evident 5 hours postadministration. Increasing inhibition occurred with increasing dose at this time point. Furthermore, inhibition of the enzymes was seen in 2 hours at the 24 mg/kg dose, and inhibition at this time point increased with increasing dose. Also, plasma cholinesterase reached maximum inhibition at 24 to 48 hours post administration as did the erythrocyte enzyme. Unlike the erythrocyte enzyme, plasma cholinesterase activity was recovered in all dose groups at the 7-day time point. Thus, plasma cholinesterase appeared slower to respond and quicker to recover than did the erythrocyte enzyme.

In a second phase of the study, groups of 5 male rats were administered NTN 8629 as before, but were sacrificed at 24 hours postadministration for brain cholinesterase assay. In this study brain cholinesterase inhibition was not seen until the 97 mg/kg dose was reached and was markedly inhibited at the higher dose. (table, p. 8).

An in vitro study of NTN 8629 inhibition of cholinesterase from all three sources of the enzyme yielded the following I_{50} values:

Erythrocyte	2.22×10^{-3} mole NTN 8629
Serum	9.2×10^{-4} mole NTN 8629
Brain	2.03×10^{-4} mole NTN 8629

Conclusions:

Thus the no effect level for plasma cholinesterase was 4.9 mg/kg, for erythrocyte cholinesterase was < 4.9 mg/kg and for brain cholinesterase was 49 mg/kg.

Core Classification: Minimum

Study: NTN 8629 - Subacute Toxicological Studies on Rat (3-Month Feeding Experiment)

Laboratory: Nitokuno, Agricultural Chemicals Institute, Laboratory of Toxicology, Toyoda

Study Number and Date: Tab 10, September 17, 1974

Accession Number: 073098

Material Tested: NTN 8629 (95% Purity)

Animals: SPF-SD Strain Rat

NTN 8629 was evaluated in a 3-month feeding experiment (actually, 17 weeks) in male and female rats. Animals employed in this study were SPF-SD strain rats bred by NIHON Clea Co., Ltd. Rats were 6 weeks old and weighed 130 grams (males) and 150 grams (females) at the inception of the study.

NTN 8629 (purity 95%) was mixed with powdered feed at concentrations of 0, 8, 40, 200, 1000, and 5000 ppm. Each dose group consisted of 10 male and 10 female rats. During the course of the study, the rats were allowed free access to food and water.

At the end of the study clinical laboratory examinations were performed on all rats of each group. These included:

a) Blood examinations: Specific gravity of whole blood, hematocrit, hemoglobin, erythrocyte and leukocyte counts and microscopic blood examination.

b. Biochemical analyses: Activities of the following enzymes were determined: glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase and lactose dehydrogenase. The following were also assayed: cholinesterase, blood urea nitrogen, uric acid, glucose, cholesterol, total protein, calcium ion, inorganic phosphorus, and albumin.

Animals were sacrificed and organs examined macroscopically and histopathologically. Results are characterized as follows: at the high dose, 5000 ppm, rats of both sexes consumed less food, and food efficiency was reduced. Similarly, slight reduction in food intake was observed at 1000 ppm. No such differences were noted for the 200 ppm group. Due to the declining food intake, there was a consequent 50 to 60 percent decline in active ingredient intake by the end of the experiment (table 1, p. 15).

At 5000 ppm piloerection and "nervous characters" were observed in both sexes, but such symptoms were not observed at doses at 1000 ppm and less.

Body Weight:

Among male and female animals, body weight was reducted for the entire 17-week period of feeding in the high-dose group. In the 1000 ppm dose groups body weights were also reduced with respect to controls, being more apparent only after 2 to 3 weeks of exposure to the treated diet (figures 1 and 2, pp. 22 and 23). No change in body weight of either sex was observed at 200 ppm.

With respect to relative organ weight data, significant increase in liver and spleen weights were noted in the high-dose male group. In the high-dose female group, there were significant increases in relative weights of brain, heart, liver, kidneys, and spleen. Also in the 1000 ppm dose group females there were increases in liver and kidney weights.

Among blood clinical analyses performed, observations were unremarkable except for leukocyte count which was modestly increased in the high-dose female groups. There were no significant differences in percent lymphocytes, neutrophils, monocytes, eosinophils, and basophils (tables 2 and 3, pp. 16 and 17).

Among biochemical parameters, total protein, albumin, A/G, and Ca^{++} were reduced in the 5000 ppm groups. Among male animals, uric acid was elevated in the 8 to 1000 ppm groups but not in the 5000 ppm group, whereas with females uric acid was elevated in all dose groups. At all doses serum GOT was reduced in male but not female rats. SGPT was reduced in male and female animals only at the 5000 ppm level of exposure. Inorganic phosphorus was elevated at the 8, 200, 1000, and 5000 ppm dose levels in females. Glucose levels were lowered in females at all doses, particularly at 5000 ppm (table 4, p. 18).

With regard to cholinesterase activity among male and female rats, plasma cholinesterase was measurably and progressively inhibited at doses of 40 ppm through 5000 ppm, the percent decrease for males being 29 percent (40 ppm), 60 percent (200 ppm), 72 percent (1000 ppm) and 79.7 (5000 ppm). For females the inhibitions at the same respective doses were 48 percent, 84 percent, 88 percent, and 91 percent. Inhibition of erythrocyte cholinesterase was similarly observed in the 40 to 5000 ppm dose range, where the inhibition was 46 percent at 40 ppm and 87 percent at 5000 ppm. In females, the respective percent inhibitions were 39 and 90. Brain cholinesterase was not significantly inhibited in either sex until the 200 ppm dose was reached, at which dose

percent inhibition for males was 16 and for females 7. At the highest dose the brain enzyme was inhibited 61 and 60 percent in males and females, respectively.

Where organ weight changes are concerned, among male animals there were no dose related or other remarkable findings with the possible exception of the submaxillary gland, the mean weight of which was significantly lower than that of the control animals, 0.711 ± 0.068 vs .859 ± 0.094, respectively. Among females, liver weight was increased in the 1000 ppm and 5000 ppm dosed animals vs control: 11.6 ± 1.3, 12.2 ± 1.16 vs. 9.99 ± 1.06, respectively. The submaxillary gland weight was also reduced in females, but the difference was less marked than in males.

Autopsy Results:

There were no autopsy records submitted.

Histopathology:

A) Males: Microscopic examination of tissues of the brain, heart, lung, and stomach did not disclose any dose related on other remarkable findings with the possible exception of the lung where bleeding was noted in 5:10 animals of the high-dose groups. Examination of the thymus disclosed moderate bleeding in three animals of the 40 ppm dose group, four animals of the 200 ppm group and one animal of the 5000 ppm dose group. Serious bleeding in the medulla area was noted in one animal of the 200 ppm dose group. Significant fatty deposition within the liver was observed in the 5000 ppm dosed animals (actual number of observations not mentioned).

B) Females: With respect to the heart, lung, liver, and kidney, there were no dose-related or other remarkable findings from among those described. There were no comments with respect to observations on the thymus gland.

It should be noted that no individual necropsy or microscopic data is provided in the study. The reviewer must rely on comments as to microscopic findings in the Results section, which appear on an organ-by-organ basis where only the number of abnormal findings are listed (p. 10 to 11). The Methods and Materials section lists organs to be examined microscopically (p. 4). The thymus gland is not included on that list yet it appears under the histopathology comments of the Results section, but only for male rats (p. 10). Was this organ not examined in females or were all females examined and found to be lesion free?

A significant point is that the histopathology results lack the thoroughness required for an adequate review.

Conclusions:

1. Only animals dosed at 5000 ppm NTN 8629 in the diet exhibited, in the words of the study director, "nervous characters" (as translated) which presumably were physical characteristics of the type generally associated with cholinesterase inhibition. Male and female rats exposed to 1000 ppm and less in the diet did not display such characteristics. Whether the "nervous characters" indicated are possibly those of neurotoxicity is unknown to this reviewer.

2. The NOEL for both plasma and erythrocyte cholinesterases was 8 ppm for both sexes. The NOEL for brain cholinesterases was 40 ppm, also with respect to both sexes.

3. Rats of both sexes experienced diminished weight gains at 1000 ppm which became marked at 5000 ppm. Animals in these groups consumed less food and consequently had diminished active ingredient intake.

4. A number of blood chemistry parameters were apparently influenced by the administration of NTN 8629. These included decreased glucose in females over the 8 to 5000 ppm dose range. This repression was marked at 5000 ppm. Increased uric acid levels were observed in both sexes through the 8 to 5000 ppm dose range. Reduced SGOT was observed in males at 8 to 5000 ppm and elevated inorganic phosphorus was observed in male rats at 8 to 5000 ppm and in females at 8 and 1000 ppm.

5. With respect to organ weight changes, the submaxillary gland displayed a decreased weight at 5000 ppm for both sexes and additionally at 1000 ppm for females. It should be noted that there was a decreased weight of the submaxillary gland observed at the high dose in mice of both sexes in the 3-month mouse feeding study submitted as a corollary to this study. There were modest but significant enlargements of the liver in females at the 1000 and 5000 ppm dosage levels.

6. There were no changes evident upon pathological examination which is considered compound-related, possibly excepting bleeding from the lungs observed in five males from the high dose group and also from the thymus gland of eight males from various dosage groups (a non dose-related finding). A suspected contaminant of chlorophenols from which NTN 8629 is produced is dioxin. This compound exerts a unique effect on the immune system including the thymus gland and spleen. Furthermore, dioxin induces hemorrhages in various organs (both of these efforts resulting from exposures to microgram quantities) [Gupta et al., (1973)]. In view of the hemorrhage phenomenon observed and particularly in the thymus gland, a full description of the test material is needed.

Comments on Conformity with Guidelines

1. Purity of NTN 8629 not discussed and must be submitted.

2. Cageside observations as described under section 82-1(g)(8)(iv)(A-G) were not reported (p. 70 guidelines).

3. Section 82-1(g)(10) indentified organs to be necropsied. Among the organs so listed, data is lacking on the following: thyroid, parathyroid, pituitary, adrenals, pancreas (important in glucose regulation), uterus, aorta, esophagus, stomach, other G.I. anatomy, bladder, lymph nodes, peripheral nerve.

4. Full histopathology data on tissues examined at autopsy are lacking.

5. A NOEL was not demonstrated.

6. Core Classification: Invalid

   Can be updated to core minimum by submitting histopathologic data as required by guidelines.

Reference

Gupta, B.N.; Vos, J.G.; Moore, J.A.; Zinkl, J.G. and Bullock, B.C. (1973) Pathologic Effects of 2,3,7,8-Tetrachloro-dibenzo-p-dioxin in Laboratory Animals. Env. Health Per. Vol. 5, pp. 125-140.

Study: NTN 8629 - Subacute Toxicological Studies on Mouse (3-Month Feeding Experiment)

Laboratory: Nitokuno, Agricultural Chemicals Institute Laboratory of Toxicology, Toyoda

Study Number and Date: Tab 9, November 5, 1974

Accession Number: 073098

Material Tested: NTN 8629 (95% Purity)

Animals: ICR-JCL Strain Mice

NTN 8629 (Purity 95%) was mixed with powdered feed in concentrations of 0, 1, 5, 25, 125, 625, and 3125 ppm. Each dose group consisted of 10 male and 10 female mice, ICR-JCL strain, bred by Nihon Clea Co., Ltd. Mice were 5 weeks old and weighed 18 grams (male) and 16 grams (female) at the inception of the study. During the course of the 3-month feeding period animals were allowed free access to food and water.

At the end of the 3-month feeding period clinical laboratory examinations were performed on all mice of each group. These included a) blood examinations: specific gravity whole blood, hematocrit, hemoglobin, erythrocyte and lymphocyte counts, and microscopic blood examination; b) biochemical analyses: activities of glutamic oxaloacetatic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, cholinesterases, and cholesterol. Animals were sacrificed and organs examined macroscopically and histopathologically.

Results are characterized as follows: at the high dose, 3125 ppm, mice or both sexes consumed less food and water than the other groups. Movement was also suppressed in the high-dose group. Piloerection was observed at the 5th and 6th day of exposure. Five animals experienced convulsions and death. Three male mice in the 625 ppm dose group displayed inactivity. This was not observed in females at this dose. Neither sex exhibited toxic signs at 125 ppm or less.

A reduction in food consumption and food efficiency was observed with both sexes in the high-dose group. Female mice were also affected at 625 ppm.

The following table reveals changes observed in clinical parameters at the various dosage levels.

Tabulation Showing Changes (%) in Parameters Indicated

	1	5	25	125	625	3125
Hemoglobin						
Male:	-*	↑**18	↑39	↑40	↑37	↑36
Female:	-	-	-	-	-	-
NOEL: Male 1 ppm, Female 3125 ppm						
Erythrocyte Count						
Male:	-	-	-	-	↓10	↓12
Female:	-	-	-	↓6	↓7	↓9
NOEL: Male 125 ppm, Female 25 ppm						
Leucocyte Count						
Male:	-	-	-	-	↑17	↑27
Female:	-	-	-	-	-	↑18
NOEL: Male 125 ppm, Female 625 ppm						
ALP Activity						
Male:	-	-	↓24	↓30	↓45	↓43
Female:	-	-	↓14	↓28	↓50	↓29
NOEL: Male, Female 5 ppm						
SGOT Activity						
Male:	-	-	↓20	↓35	↓21	↓19
Female:	-	-	↓20	↓33	↓29	↓30
NOEL: Male, Female 5 ppm						
GPT Activity						
Male:	-	-	-	↓42	↓40	↓5
Female:	-	-	-	-	-	-
NOEL: Male 25 ppm, Female 3125 ppm						

* (-) Signifies no meaningful change
** (↑) Signifies increase, (↓) decrease

Cholinesterase inhibition was very evident. Significant inhibition was seen for plasma cholinesterase at $\geq$ 5 ppm for males and females, NOEL 1 ppm. The erythrocyte enzyme was significantly inhibited in males at $\geq$ 25 ppm, NOEL 5 ppm, but in females, while the study director indicates significant inhibition at dose of $\geq$ 5 ppm, the data appear to indicate a dose response phenomenon first detectable at the lowest dose, 1 ppm (table 5). Our independent t-test calculations show the erythrocyte cholinesterase value for females at 1 ppm is significantly different ($p < .05$) from the control, hence, NOEL < 1 ppm. Furthermore, the data point appears consistent with a dose response. Brain cholinesterase in males is significantly inhibited at 125 ppm, NOEL 25 ppm. In females there is an observable dose response effect apparent from the lowest to the higest dose, significant at 25 ppm. There is the suggestion of an effect at lower doses. From the standpoint of statistical significance, NOEL for females is 5 ppm. See table of cholinesterase data.

Modified Table 5 In Study Report
Cholinesterase activity in Δ pH
% = % Inhibition, For Statistically Significant Changes

Dose in ppm	Plasma $\bar{x}$	Plasma σ	Plasma %	Erythrocytes $\bar{x}$	Erythrocytes σ	Erythrocytes %	Brain $\bar{x}$	Brain σ	Brain %
MALE									
0	1.56	0.243		0.35	0.046		1.37	0.153	
1	1.73	0.234		0.33	0.042		1.34	0.065	
5	1.10**	0.336	29	0.28	0.059		1.36	0.089	
25	0.42**	0.062	73	0.10**	0.026	71	1.35	0.047	
125	0.29**	0.030	81	0.05**	0.014	86	1.17**	0.050	15
625	0.30**	0.011	81	0.05**	0.016	86	0.75**	0.057	45
3125	0.25**	0.014	84	0.05*	0.017	86	0.52**	0.022	62
FEMALE									
0	2.14	0.128		0.42	0.068		1.58	0.122	
1	2.28	0.133		0.37*	0.047		1.52	0.121	
5	1.79**	0.197	18	0.32**	0.085	24	1.48	0.099	
25	0.47**	0.042	78	0.15**	0.014	64	1.41**	0.105	11
125	0.26**	0.023	88	0.07**	0.025	83	1.27**	0.095	20
625	0.20**	0.030	91	0.05**	0.012	88	0.83**	0.063	47
3125	0.25**	0.100	88	0.05**	0.0004	88	0.40**	0.028	75

* Significant differences in comparison with control group ($p < 0.05$)
** Significant differences in comparison with control group ($p < 0.01$)

Organ weight changes were unremarkable except for a significant reduction in the submaxillary gland and an increase in adrenal gland size in the male 3125 ppm group. Similarly, for females, adrenal weight was increased in the high-dose group. A decline also appears for the submaxillary gland among the female high dose groups. Notably, spleen and thymus were not affected at any dose level (table 6).

When expressed on a relative organ weight basis only, liver exhibited an upward trend with dose which was most marked in the 3125 ppm group of both sexes. There were other scattered statistically significant findings but none showing a consistent or dose response relationship. The submaxillary gland was decreased down in females at the high dose (table 7).

In general histopatholgic findings do not cover any dose-related trends or other remarkable effects, with the exception of the adrenal gland response. With respect to the adrenal the study author indicates that vacuole degeneration was observed in many animals of the control and dose groups. Data on these findings are not present and, hence, review is not possible.

Deficiencies: The study does not provide individual animal data or histopathologic observations on individual bases. Inadequate information on strain of mouse employed.

Significant Findings

1. Cholinesterase NOEL:

   Plasma, male = 1 ppm
   female = 1 ppm

   ER, male: 1 or 5 ppm
   female: < 1 ppm

   Brain, male: 25 ppm
   female: 5 ppm

2. No-effect level for food consumption and weight gain for males and females appears to be 125 ppm.

3. Liver enlargement was detected in male and female mice at 5 ppm, a dose-related phenomenon.

4. Hemoglobin was significantly elevated at $\geq$ 5 ppm in male mice.

5. Due to the lack of adequate individual histopath data, etc., the study must be considered core supplementary.

   Core Classification: Supplementary

Study: 6-Month Feeding with 1-Month Recovery on Rats

Laboratory: Nitokuno, Agricultural Chemicals Institute, Laboratory of Toxicology, Toyoda

Study Number and Date: Tab 11, May 20, 1977

Accession Number: 073098

Material Tested: Prothiophos

Animals: Rat

Introduction

The intent of the study was to evaluate histopathological responses in a number of selected organs of the rat following administration of prothiophos for a 6-month period, and then to reevaluate after 1 month of recovery following discontinuation of administration of the compound.

Procedure

The study is deficient in that it does not contain any methods and materials section, and only includes the slight procedural information that can be gleaned from various sections of the report. These include:

1. Apparently rats in groups of 10 male and 10 female per dose were exposed to prothiophos at 0, 5, 50, 500, and 5000 ppm in the diet for 6 months.

2. Perhaps half of these, or another fraction thereof, or yet other groups were exposed for 6 months followed by a 1-month recovery period.

3. Selected tissues including: brain, thyroid gland, thymus, heart, pancreas, stomach, large intestine, adrenal gland, gonads, skeletal muscle, pituitary gland, submaxillary gland, lung, liver, lymph nodes, small intestine, kidney, urinary bladder, spleen and bone marrow were examined histopathologically after 6 months and then in other rats after the 6-month exposure plus 1-month recovery period.

Comment - No other experimental protocol is provided. There is no indication of the source of prothiophos used. There is no description of the manner of administration.

Results

I. Feeding

The only structures evidencing an effect were liver, testis, adrenal, bone marrow, and intestine. According to the study author, only effects on the liver were considered to be toxicologically significant. These included fatty degeneration in the central lobule, swelling, nuclear pleomorphism, nuclear degeneration, single cell necrosis, bile duct proliferation, and pigment deposit in Kupffer cell. The incidence and degree of these changes were observed at 500 and 5000 ppm in a dose-related manner.

Changes not veiwed as compound related were the atrophy of the testis in one male at 50 ppm, small cell nodule of the adrenal in one male at 50 ppm, a decrease of erythrocytes in the bone marrow of one male at 5000 ppm, and edema of the small intestine in one female at 5000 ppm.

II. Recovery

The study author indicates minimal hepatic lesions were observed in male and female animals at the two high doses. However, there were fatty degeneration in the control lobule, swelling, nuclear pleomorphism, nucleus degeneration, and single cell necrosis. These finding were dose related.

Additional observations were variable and generally unremarkable.

Conclusion

The conclusion reached by the study author was that in rats dosed at 500 and 5000 ppm minimal to moderate hepatic changes occurred. These changes were considered induced by the compound and were "remarkably related with the dosage."

"However, a slight reparation of these changes were found within a 1 month-recovery period, and it is found that these hepatic lesions could be reversible changes" p. 3. (Note: it is possible that something is lost in translation here.)

This reviewer's comments are as follows:

1. There are hepatic effects of the compound, regardless of how administered, at 500 and 5000 ppm, but according to information on page 5 and 6, such effects are not induced at the lower doses, hence, the NOEL for hepatic effects would appear to be > 50 ppm.

2. The nuclear pleomorphism and nuclear degeneration are reminiscent of the spermatogonial karyopyknosis observed in the multigeneration study (volume 2, tab 3, 3d set), and as reported to occur in other tissues (spleen, bone marrow, and renal tubule) in response to TCDD [Gupta, et al. (1973)].

3. In the initial feeding study, there was one rat in the 50 ppm dose group exhibiting minimal testicular atrophy with aspermatogenesis (p.5).

In general, study details are lacking. There is no description as to the source or quality of the test material and there are no histopathologic result sheets, only summaries. Many different tissues were examined, and to accept a mere statement of negative findings in the bulk of these tissues would not be consistant with the Agency's requirements. Detailed examinations of thymus, testis, spleen, lymph nodes, bone marrow, etc. is mandated by findings in other studies. This study means very little.

Core Classification: Invalid

Could be updated to supplementary by submission of histopathologic data.

Reference

Gupta, B.N., Vos, J.G., Moore, J.A., Zinkl, J.G. and Bullock, B.C. (1973): Pathologic Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Laboratory Animals. Env. Health Perspectives, 5 p. 125.

Study: Tumorigenicity Study in the Rat

Laboratory: Institute of Comparative and Human Toxicology, Albany Medical College, Albany, NY and International Center of Environmental Safety, Albany Medical College, Holloman AFB, New Mexico.

Study Number and Date: TAB 15, June 29, 1978

Accession Number: 073092

Material Tested: NTN 8629 (93.2%, Bayer, AG); Prothiophos

Animals: Long-Evans Rat

IA. Methods and Materials (Quoted from Submission, pp. 002-003)

Test Compound: A sample of technical grade NTN 8629 (Sdg. 1604/74, 93.2%) was used in the study. The material was supplied by the Bayer A.G., as a premix with the inert material Wessalon S (synthetic silica) 1:1.

Experimental Animals and Their Maintenance

Eight hundred and forty pathogen-free CD (Long-Evans) rats were acclimated to laboratory conditions and were divided into five groups in which the animals had approximately equal body weights. One of the groups was fed the carrier substance Wessalon S (provided by Bayer A.G.) in a concentration of 500 ppm, the same concentration fed those animals receiving the highest concentration of NTN 8629. The ages of males and females at the beginning of the study were 52 and 56 days, respectively.

B. Protocol:

1. Study Design

The dosage groups were:

Group	Dietary Level (ppm)	No. of Animals Male	Female
1	1	105	105
2	5	70	70
3	50	70	70
4	500	105	105
5	500 of Wessalon S	70	70

The first sixty male and female rats in each group made up the main experimental groups which were used primarily for carcinogenic evaluation. They were not used for provision of blood samples except at the termination of the study or due to absolute necessity. The remaining animals in each group made up the satellite groups which were used for provision of blood samples and were not included in the carcinogenic evaluation. These rats were discarded after blood sampling at week 104; an autopsy was not performed.

The animals were maintained in air-conditioned quarters with food and water available ad libitum and were housed singly in polypropylene cages providing 956 sq cm of floor space (Laboratory Products, Inc., No. 18580). The diet was Wayne Lab-Blox Mash.

Dietary mixtures were prepared once a week in a Hobart laboratory mixer, and assayed immediately to assure that the desired concentration limits for each group were not exceeded.

Blood samples at 0, 2, 4, 6, 8, 13, 26, 52, and 78 weeks were obtained from the orbital sinus of anesthetized (Halothane) Satellite animals and the animals were returned to the colony. Blood samples at week 104 were obtained from the abdominal aorta of anesthetized (Pentobarbital sodium, 30 mg/kg, i.p.) animals by means of a 21 or 22 gauge needle and a 10 mL syringe.

Feeding of NTN 8629 was begun on February 20, 1976. The study was terminated on March 7, 1978. It is noted at this point that a failure of the air-conditioning system resulted in the death of 264 animals in the various groups due to external systemic hyperthermia.

2. Parameters Examined

Toxic signs and symptoms, body weight, food and water consumption, ophthalmoscopy, hematology, blood chemistry, urinalysis, cholinesterase (plasma, erythrocyte, brain), organ weights, gross necropsy, histopathology.

According to the report animals were observed daily for symptoms of toxicity and palpable tumors. All animals found dead were stored at 16 °C and autopsied as soon as possible. The authors described the signs of toxicity to be similar to those observed in the rat multigeneration study. These included, at the high dose (500 ppm), cholinergic effects such as hyperactivity, tremors, and hypersensitivity

to sound appearing after 7 to 11 weeks of exposure, females being more markedly affected. Other signs of toxicity according to authors were aggressivity, vocalization upon handling and hind leg weakness. Piloerection was also observed. Such signs were not observed among animals fed the 50 ppm NTN 8629 diet.

Reviewer's Discussion and Interpretation of Study Results:

A. Toxic Signs

The study authors commented that the toxic symptoms observed were similar to those observed in the multi-generation study. Signs of cholinesterase inhibition were noted in the high-dose group, but in addition the study reports signs of aggressivity, vocalization upon handling, and hind leg weakness. These latter signs may also be due to cholinesterase inhibition, but could have yet another neurologic etiology. These observations reinforce the need for a neurotoxicity study on NTN 8629.

B. Body Weight

The essential points to be made from data submitted on body weights (graphs p. 1, tables 1 and 2, pp 2 to 9) are that effects of NTN 8629 on body weight gain of male rats were not marked at any dose, although there were slight variations with respect to controls at various dose and time intervals. All males in groups 1 (control) and 2 (5 ppm) were lost after the 55th week.

Among female rats, there was a consistent significant repression of weight gain in the high-dose group throughout the 104 weeks of observation. Among females in the mid-dose group (50 ppm), significant increases of body weight were observed during certain time intervals, for example weeks 37 to 53 (pp 7 to 8) and 76 to 93 (pp 8 to 9).

C. Food and Water Consumption

In male rats, food consumption data did not show any consistent or dose-related influence of the test compound. In high-dose female rats there was a significant increase in food consumption of the during the first 13 weeks of study, but this normalized during the remaining study interval.

It is significant to note, therefore, that the repressed body weight increase noted among females in the high-dose group occurred in the absence of food intake inhibition. This, in addition to the periodic

increases in body weight among females of the 50 ppm group suggests an influence of NTN 8629 on metabolism or food processing. Negative influence on weight gain in the rat is a characteristic of TCDD (expected contaminant of chlorinated phenols) exposure (Vos, et al. 1973). Other characteristics of TCDD exposure, such as effects on the thymus, spleen, adrenal, lymphoid system, and liver will be commented upon subsequently.

It should be noted that intake of NTN 8629 in terms of mg/kg/day was higher in female rats at all three dose levels, due to relatively lower body weight rather than increased food intake as compared to these parameters in males (p. 10).

D. Ophthalmoscopy

There were more animals exhibiting opacity in the 500 ppm dose group than in the control groups. Whether the small difference (2 control vs 7 treated rats) represents a toxicologic response is uncertain. It is known that in diabetes there is an increased incidence or cataract formation. Glucose was elevated in high-dose females at 78 and 104 weeks (see blood chemistry) and five of the seven high-dose rats with opacities were females.

E. Hematology

Tables 5, 6, 7, 8, and 9 are cited as illustrating hematologic comparisons between control and 500 ppm dose groups at 0, 5, 13, 25, and 52 weeks. Although data in the tables cited do not cover precisely these time intervals, and legends on table 5 (p 27) and 6 (p 43) are in Japanese, there do not appear to be any remarkable effects on the hematologic parameters.

F. Blood Chemistry

Parameters examined include Na, K, glucose, Bun, total protein, albumin, SGOT, SGPT, ALP, and cholesterol. The only remarkable findings were: 1) repressed alkaline phosphatase (ALP) in the high-dose male group at weeks 78 and 104. The same enzyme was also reduced (not with statistical significance) in the middle-dose group (50 ppm) at 104 weeks; 2) elevated blood glucose in females at 78 and 104 weeks, an observation not cited by study authors.

G. Urinalysis

Parameters examined included specific gravity, pH, protein, glucose, ketone, bilirubin, urobilinogen, and total reducing substance. There do not appear to be any remarkable findings.

H. Cholinesterase

Plasma cholinesterase inhibitions (mean) over the 104 week period were 47% (male) and 48% (female) in the 50 ppm dose group, and 84% (male and female) in the 500 ppm dose group. The NOEL (Plasma ChE) was 5 ppm for both sexes. RBC cholinesterase inhibitions (mean) over the 104-week period were 30% (male and female) in the 50 ppm dose group and 53% (male) and 45% (female) in the 500 ppm dose group. The inhibition among female rats was not first apparent until the 8th week of study in the 50 ppm dose group. The NOEL (RCB ChE) for both sexes was 5 ppm. Brain cholinesterase was inhibited 63% (male) and 66% (female) in the 500 ppm dose group as measured at the end of the study. Inhibition at lower doses was not seen; NOEL (brain ChE) was 50 ppm for both sexes.

I. Histopathology - Tumorigenicity

The study report indicates that after 12 to 13 months into the study a failure of the air-conditioning system resulted in the death of 264 animals. At the time of the accident it was decided to continue the study, but also to initiate a new study. Apparently it was also decided to save tissues only from terminally sacrificed animals for microscopic examination (page 173). Thus all animals which died during the air-conditioning failure, those additional rats which subsequently died spontaneously, and those which were sacrificed early were not examined histologically.

In the following table the number of rats in each group at the beginning of the study is indicated under column A, while the number of rats surviving for histopathologic examination is indicated under column B.

	Male		Female	
Group	A	B	A	B
I	105	0	105	32
II	70	0	70	29
III	70	12	70	41
IV	105	11	105	20
V	70	42	70	40
Total	420	65	420	162

The number of animals which died as an immediate consequence of the air conditioning failure was, as indicated above 264, however, the total number of animals lost as far as histopathologic examination is concerned was according to the above table 840-227 = 613. Thus, in the course of the study following the time of air conditioning failure a large number of animals died prematurely or were sacrificed early. Since after the air conditioning failure the decision was made to examine histologically only those animals reaching terminal sacrifice, apparently little care was taken to carefully store and examine animals not reaching terminal sacrifice and this we surmise accounts for the large number of rats reported as autolyzed. In view of the fact that histologic data were reported only for terminally sacrficied animals, the data are biased.

A consolidated tumor count, i.e., combination of confirmed tumors from histopathology sheets with those identified on autopsy sheets is presented in the following table 1.

Legend For Table 1:

A. Number of rats with tumors from histopathology sheets of terminally sacrificed animals.

B. Number of rats with tumors from autopsy sheets of terminally sacrificed animals, [the number in parentheses ( ) is the number of rats among these with no reported tumor on histopathology sheets].

C. Number of rats with tumors (as identified from autopsy sheets) which did not reach terminal sacrifice.

D. Sum of A, B( ), and C.

Table 1

		A	B	C	D	Number of rats out of 60 per group with at least minimal data on histopathology and/or autopsy sheets
Group I	(0 ppm)					
	M	--	--	--	--	--
	F	22	9(0)	5	27	49
Group II	(5 ppm)					
	M	--	--	--	--	--
	F	15	9(2)[V]	12	29	57
Group III	(50 ppm)					
	M	4	1(1)[W]	5	10	24
	F	28	12(3)[X]	11	42	60
Group IV	(500 ppm)					
	M	6	5(0)	3	9	20
	F	7	2(0)	13	20	60
Group V	(carrier)					
	M	21	9(2)[Y]	11	34	60
	F	26	12(3)[Z]	7	36	60

Rat ID Numbers: [V](320, 339), [W](396), [X](429, 474, 478), [Y](721, 736), [Z](789, 794, 805).

Histopathology data were not reported for all tumors identified on autopsy. Most of the deficiency is with respect to animals that did not reach terminal sacrifice, although some tumor incidences (i.e., gross necropsy) of terminally sacrificed animals are not addressed in the histopathology sheets.

Assuming only those tumors characterized by histopathic examination are real, the proportion of such tumor-bearing animals to the total number of animals which reached histopathic examination is as follows:

Animals with Tumor (by Histopathic Exam)/Animals Histopathed

Group	I	
	F	22/32 = 68.8%
Group	II	
	F	15/30 = 50.0%
Group	III	
	M	4/12 = 33.3%
	F	28/41 = 68.3%
Group	IV	
	M	6/11 = 54.5%
	F	7/20 = 35.0%
Group	V	
	M	21/42 = 50.0%
	F	26/39 = 66.7%

A tumorigenic trend is not evident. This is true not only as shown here in terms of absolute numbers of tumor-bearing animals, but is true in terms of the tissue site tumorigenic response as revealed by histopathologic examination. However, in view of the large number of animals either lost in the study, or for which no histopathologic data apparently exists (or was reported), it is not surprising that a trend is not identified. This study is wholly inadequate to identify a tumorigenic response. In support of this we note that:

1. For male animals no data exists for Group I and Group II animals. Data is wholly insufficient for Group III and Group IV males, i.e., 36 and 40 blank autopsy sheets exist in these groups, respectively. Thus, male data in this study are virtually useless for an oncogenic evaluation.

2. For female animals, in Group I only 32 animals were examined histopathologically and in Group II only 30; it is not clear whether these animals suffered in any way from the failure of the ventilation system. In Group III, there were 41 examined histopathologically. There were 3 animals apparently with some sort of growth at autopsy which were not examined histopathologically, but which if counted would yield 31 animals with tumor, a high number for only 41 animals histopathed (or 44). For Group IV females, only 20 animals were histopathed, a large number being lost to autolysis.

Conclusions:

1. Rats of both sexes receiving 500 ppm NTN 8629 in the diet exhibited significant inhibition of body weight increase. This was more evident and consistent in the case of females.

2. Male rats experienced periodic increases in growth rate when receiving 50 or 5 ppm NTN 8629 in the diet.

3. The NOEL for weight gain in female rats was 5 ppm NTN 8629.

4. Food consumption was not remarkably influenced by NTN 8629 at any dose.

5. Negative effects on body weight gain observed in females receiving the high dose, without any apparent effect on food consumption, might be viewed as a toxic effect of NTN 8629 on metabolism, food utilization, nutrition, etc. [Note: These same effects are indicative of TCDD toxicity. TB is therefore requiring an analysis of dioxin content in the test material used in this study.]

6. Animals receiving the diet containing 500 ppm NTN 8629 exhibited toxic signs including hyperactivity, tremors, hypersensitivity to sound, aggressivity, vocalization on handling, hind leg weakness, and piloerection. No toxic signs were found at 50 ppm NTN 8629. The signs observed may be due solely to cholinesterase inhibition, however, certain of these symptoms may be due to other effects on its nervous system and reinforce the need for a neurotoxicity study.

7. Hematologic parameters were not significantly altered at any dose level. Among blood chemistry parameters alkaline phosphatase was depressed at certain time points in the high-dose male group and was numerically (not statistically significantly) reduced in the middle dose (50 ppm) group at 104 weeks. Blood glucose was elevated in females at 78 and 104 weeks. There was a slightly increased number of eye opacities noted in the high-dose female.

8. The NOEL for plasma and erythrocyte cholinesterases in males and females is 5 ppm NTN 8629 in the diet. For brain cholinesterase, the NOEL in both sexes is 50 ppm in the diet.

9. The assessment of tumorigenicity cannot be determined. Too many animals were lost, both due to an air conditioning failure and to premature deaths for which no histopathology data exists, to permit a valid assessment.

   This study provides limited information about the toxicity of NTN 8629, but should be viewed as invalid with respect to assessment of tumorigenicity.

10. A complete chemical analysis of the composition of the NTN 8629 used in this study is required.

11. Core Classification - Invalid

Study: Tumorigenicity Study in the Rat II

Laboratory: Institute of Comparative and Human Toxicology, Albany Medical College, Albany, NY and International Center of Environmental Safety, Albany Medical College, Holloman AFB, New Mexico.

Study Number and Date: Volume 2, Tab 16, December 21, 1979.

Accession Number: 073092

MRID:

Material Tested: NTN 8629 (93.2%, Bayer AG)

Animals: CD Long-Evans Rats, Young Males and Females

I. Methods and Materials

"a. Test compound: A sample of technical grade NTN 8629 (Sdg. 1604/74, purity of 93.2%) was used in the study. The material, supplied by the Bayer AG as a premix with the inert material Wessalon S (synthetic silica) 1:1, was fed from April 15, 1977 to May 2, 1979.

"b. Experimental Animals and Their Maintenance: Five hundred pathogen-free CD Long-Evans rats (Blue Spruce Farms, Altamont, NY 12009) were acclimated to laboratory conditions and were divided into three groups in which the animals had approximately equal body weights. The ages of males and females at the beginning of the study were 50 and 55 days, respectively, with a weight range of 116 to 174 g for males and 126 to 170 g for females.

"The dosage groups were:

Group	Dietary Level (ppm)	Animal Identification Male	Female
1	0	M1 - M90	M251 - M340
2	5	M91 - M160	M341 - M410
3	25	M161 - M250	M411 - M500

"All animals in the study were identified by a tag clipped to an ear.

"The first 60 male and female rats in each group made up the Main experimental groups, were used primarily for carcinogenic evaluation, and not for provision of blood samples except at the termination of the study or when absolutely necessary. The remaining animals in each group made up the Satellite groups which were used for blood samples but were not included in the carcinogenic evaluation. These rats were discarded after blood sampling at weeks 104 and 106.

"Blood samples at 0, 2, 4, 5, 9, 12, 26, 52, 78, and 83 weeks were obtained from the orbital sinus of anesthetized (Halothane) Satellite animals and the animals were returned to the colony. Blood samples at 104 and 106 weeks were taken from the abdominal aorta of anesthetized (Pentobarbital sodium, 30 mg/kg, i.p.) animals by means of a 21 or 22 guage needle and a 10 mL syringe.

"The animals were maintained in air-conditioned quarters with food and water available ad libitum and were housed singly in polypropylene cages providing 956 sq cum of floor space (Laboratory Products, Inc., No. 18580). The diet was Wayne Lab-Blox Mash.

"Dietary mixtures were prepared once a week in a Hobart Laboratory mixer, and assayed immediately to assure that the desired concentration limits for each group were not exceeded." (pp. 3 to 4)

c. Parameters Examined: Toxic signs and symptoms, body weight, food and water consumption, hematology, blood chemistry, urinalysis, cholinesterase (erythrocyte, plasma, and brain) ophthalmoscopy, distribution of mortality, organ weights, gross necropsy, histopathology.

II. Results:

a. Toxic signs and symptoms: There were no adverse effects with regard to physical appearance or behavior for either sex at the two dose levels studied.

b. Distribution of mortality: Mortality over the 24 months of study was unaffected by the 5 and 25 ppm dosing regimens in the Main group and for females of the Satellite group. Males in the Satellite group were adversely effected in the high-dose group. At 24 months the percent mortality for the 0, 5, and 25 ppm groups was 57, 50, and 70 percent, respectively.

c. Body weight: Boby weights were generally unaffected by treatment.

d. Food and water consumption: Food consumption was significantly elevated in the high-dose male group at weeks 17 to 18, 20, 23 to 26, and 28 to 30 reduced at weeks 40 and 43, and then normalized. Among females there were no remarkable compound-related effects (tables 3 and 4).

e. Ophthalmoscopy: Eye examinations of rats of both sexes did not disclose any particularly meaningful findings.

f. Hematology: Hemoglobin, packed cell volume, red blood cell count, white blood cell count and prothrombin time were not affected in any consistent manner in animals of either sex of the high-dose group. Data are not tabulated for the low-dose (5 ppm) group (table 6). (tables 7 and 8).

g. Blood chemistry: Results do not reveal any consistent dose-related effects.

h. Urinalysis: Urine samples from the control and 25 ppm dose groups were evaluated at 7 time points (0, 5, 12, 26, 52, 78, and 104 weeks) over the 104-week period of study and no remarkable dose-related effects were observed.

i. Cholinesterase: Results of assays for plasma cholinesterase revealed slight inhibition in males dosed at 25 ppm during the limited time interval of 2 to 26 weeks (at 26 weeks inhibition was 40%), but was unchanged at weeks 78 and 106. There was no inhibition among males of the 5 ppm dose group. In females the inhibition at 25 ppm was more marked and was significant at all time points except at weeks 4 and 78 (at 26 weeks inhibition was 60%). No inhibition was seen in the 5 ppm dose group (tables 13 and 14 pp. 140, 141).

Erythrocyte cholinesterase among male rats of the 25 ppm dose group was significantly inhibited at the 9 (20%), 12 (25%), and 52 (34%) week time points. Among females at the same dose, significant inhibition was recorded at the 26 (25%) and 52 (28%) week intervals. The enzyme was not inhibited in rats of either sex in the 5 ppm dose group (tables 15 and 16).

No effect was observed on brain cholinesterase in either sex in any dose group.

It is concluded that the NOEL level in the diet for this organophosphate is 5 ppm (males and females) for plasma and erythrocyte cholinesterase, and 25 ppm (males and females) for brain cholinesterase.

j. Organ Weights: The organs weighed were liver, heart, spleen, adrenals, gonads, brain, pituitary, and kidneys. Among males, only one statistically significant change was observed which was an increased kidney weight in the high-dose group as expressed both in terms of absolute and relative organ weight. Among female rats there were no remarkable findings. Thymus weight was not determined.

k. Gross Necropsy and Histopathologic Examination: Tissues examined include heart, lung, thymus, liver, spleen, pancreas, stomach, small and large intestine, mesenteric lymph node, testis/ovary, urinary bladder, adrenals, pituitary, kidneys, salivary gland, thrachea, esophagus, thyroid, parathyroid, eye, and central nervous system. The number of rats in each group found to have tumors is reported in tabular form as follows:

Table 1

Dose	Number Rats Examined Histopathologically	Number Rats With Pituitary Tumor	Number Rats Inadequately Examined For Pituitary Tumor	Fraction of Rats Properly Examined With Pituitary Tumor
Control				
female	57* (4 missing)	18	21	18/36
male	56 (4 missing)	11	20	11/36
5 ppm				
female	59 (1 missing)**	26	27	26/32
male	56 (4 missing)	12	24	12/32
25 ppm				
female	58* (3 missing)	23	22	23/36
male	58 (2 missing)	12	31	13/27

* Includes one rat counted twice - i.e., same ID No., but separate histopath sheets. For the control, the one in question is No. 263 and for the 25 ppm group, the one in question is No. 458.

** To be evaluated, No. 363.

All listed as missing are in fact lost - see p. 177, V. 5, Tab K-2. Descriptions (nomenclature) for the various types of tumors identified are included in the table of tumor incidence found on pp. 182 through 187, V. 2, Tab 16.

In reviewing histopathology sheets and the Petition's Incidence and Type of Tumors Found in Rats Fed NTN 8629 (p. 180), the following additional pertinent observations emerge: 1) a high frequency of pituitary tumors, 2) a possible dose-related increase in pheochromocytoma of the adrenal gland, 3) a numerical increase of thyroid adenocarcinoma in the middle dose group only, and 4) a possible increase in the high-dose group of rats with carcinoma and adenocarcinoma of the lung.

A general observation among all groups was the high frequency of pituitary tumors, particularly chromophobe adenoma. This did not appear to be dose related. It was noted that in a number of cases, the slides for the pituitary were, for a variety of reasons, inadequate for obtaining histopathological information. Thus, as shown in table 1, in addition to counting the number of rats with pituitary tumors in each group, a count was taken of the number of slides which were inadequate for histopathologic examination of the pituitary. The ratio of the number of rats with pituitary tumors to the number of animals adequately examined for pituitary tumors was determined for each group and recorded as the last column in the table. As viewed in this manner, there was no dose-related increase in the incidence of rats with pituitary tumors. The high frequency of pituitary tumors may be a geriatric response.

The slight numerical increase in total pituitary tumors seen in table 1 for the 5 and 25 ppm groups, may well be largely accounted for by the findings of four rats in each of the dose groups with the malignant tumor, chromophobe adenocarcinoma. None of this tumor type were reported among pituitary tumors of the control. Whether the finding of these few malignant tumors in the dosed animals is related to NTN 8629 dosing is uncertain. Historical control data for the occurrence of this pituitary tumor in the CD Long-Evans rat is required.

With regard to adrenal pheochromocytoma, the following tabulation is applicable (combined data from information tabulated on page 180 and 184 of the study).

Table 2

Number of Rats with Medullary Adrenal Tumors

	Control	5 ppm	50 ppm
Male	5	22	10
Female	5	10	15
Total	10	32	25

Data presented in table 2 indicate a dose-related increase in tumors of adrenal medulla, an effect exhibiting dose response in females. However, the particular tumors may have a high spontaneous incidence. Historical control data will also be necessary for proper assessment of this finding.

In the case of thyroid tumors, there was an increased number of tumors in the low-dose group, but not in the high-dose group. The petitioner should be required to differentiate between follicular cell and "c" cell thyroid tumors and report the numbers of each. A trend in one or the other of these tumors may be evident when this distinction is made. Also, historical control data must be submitted.

Lastly, six rats with either carcinoma or adenocarcinoma of the lung were found in the 25 ppm dose group, as compared with but one in each of the control and 5 ppm groups. Historical control data must be submitted for the lung tumor types found.

The petitioner should be advised to submit historical control data for incidences of the following tumors in Long-Evans rats from the same lab as well as other labs: pituitary chromophobe adenocarcinoma, adrenal pheochromocytoma, thyroid adenocarcinoma, and lung carcinoma/adenocarcinoma.

There is a concern for this study in terms of the extent of autolysis. The following tabulation shows the number of animals of each group which, in the opinion of this reviewer, were autolyzed to an extent which would render questionable the diagnostic capability of histopathology.

Autolyzed Animals, %

	0 ppm	5 ppm	25 ppm
Male	20	33	30
Female	23	15	15

The total number of animals/group at the beginning of the study was 60. The above number represents considerably more than a 10 percent loss in each group. The overall loss is about 23 percent.

An additional observation to be reported from this study is the frequent occurrence of nephritis and thyroidization of the kidney. The frequency of these observations is tabulated as follows:

Kidney Observations (Number of Rats)

	Nephritis	Thyroidization	Normal	Inadequate Kidney Information
Male				
0 ppm	46	2	1	8
5 ppm	47	-	1	8
25 ppm	23	28	2	6
Female				
0 ppm	36	4	2	15
5 ppm	34	1	2	22
25 ppm	41	9	1	7

Thyroidization is defined in Dorland's Medical Dictionary, 25th ed., as ". . . the thyroid-like appearance of a tissue." As shown above, it is evident that a morphologic change occurs in male kidneys at the high-dose level.

As it may relate to this observation, Gupta et al. (1973), indicate that in the rat, exposure to dioxin is associated with pyknosis and degenerative changes in the connecting and collecting renal tubules, p. 133.

In view of the established relationship between dioxin exposure and degenerative changes in the thymus (Ref. Faith and Moore (1977)), an attempt was made to evaluate changes in the thymus with regard to exposure to NTN 8629 since dioxin contamination is a possibility. Unfortunately, there was extensive atrophy or missing thymus tissue from histopathology samples, this being true for control as well as for dosed animals. Therefore, it was not possible to determine whether NTN 8629 affected the thymus in this study.

Conclusion:

1. EPA Guidelines indicate that animals lost due to autolysis, cannibalization, etc., should not exceed 10 percent. The overall loss due to autolysis alone in this study was approximately 23 percent. In addition, approximately 5 percent of the animals are simply reported as missing. Hence, there are inadequate data on 28 percent of the animals.

2. No signs of toxicity, evidenced by physical appearance and behavior, were noted.

3. No adverse effects on body weight were observed other than an increasse in weight among males of the high-dose group during weeks 18 to 32.

4. Food consumption among high-dosed males was elevated at weeks 17 to 18, 20, 23 to 26, and 28 to 20; was reduced at weeks 40 and 45; and normalized at other times. Food consumption among females was not remarkably altered.

5. No consistent dose-related effects were observed with respect to the variety of blood chemistry, hematology or urine parameters analyzed.

6. The no-effect level for inhibition of plasma and erythrocyte cholinesterases was 5 ppm in both sexes. The no-effect level for brain cholinesterase was 25 ppm in both sexes.

7. Organ weight assessments disclosed a significant increase in kidney weight among high dosed males. This was true both with respect to absolute and relative kidney weights. There were no other remarkable organ weight findings.

8. Thyroidization of the kidney was marked among high-dosed males and apparently increased, though less frequently, among high-dosed females. This appears to be an NTN 8629 related effect. The no effect level for this phenomenon was 5 ppm.

9. For thyroid tumors petitioner should distinguish between numbers of follicular and "c" cell tumors observed in the study.

10. Nephritis was widely observed among control and dosed animals.

11. Due to the finding of a numerical increase in numbers of rats in dosed groups with pituitary chromophobe adenocarcinoma, pheochromocytoma, thyroid adenocarcinoma, and lung carcinoma/ adenocarcinoma, historical control data for these tumor types in the rat species in question must be provided.

Core Classification: Invalid

References

Gupta, B.N. et al. (1973) Pathologic Effects of 2,3,7, 8-tetrachlorodibenzo-p-dioxin in Laboratory Animals. Env. Health Perspectives, Vol. 5, pp. 125-140.

Faith, R.E.; Moore, J.A. (1977) Impairment of Thymus-Dependent Immune Functions by Exposure of the Developing Immune System to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). J. Tox. Env. Health 3:451-464.

Study: Tumorigenicity Study in the Mouse

Laboratory: Institute of Comparative and Human Toxicology, Albany Medical College, Albany, NY and International Center of Environmental Safety, Albany Medical College, Holloman AFB, New Mexico.

Study Number and Date: Volume 1, Tab 14, September 1, 1978.

Accession Number: 073092

MRID:

Material Tested: NTN 8629 (93.2%, Bayer, AG)

Animals: Charles River Mice

I. PROCEDURES

A. Methods and Materials (Quoted from Study Report, pp. 2-3)

"Test Compound: A sample of technical grade NTN 8629 (Sdg. 1604/74, 93.2%) was used in the study. The material was supplied by the Bayer AG, as a premix with the inert material Wessalon S (synthetic silica) 1:1.

Experimental Animals and Their Maintenance

Eight hundred and forty Charles River CD-1 mice were acclimated to laboratory conditions and were divided into five groups in which the animals had approximately equal body weights. One of the groups was fed the carrier substance Wessalon S (provided by Bayer AG) in a concentration of 400 ppm, the same concentration fed those animals receiving the highest concentration of NTN 8629. The age of males and females at the beginning of the study was 57 days." (Note: Source and strain of mouse not provided, p. 2.)

B. Protocol

1. Study Design

"The dosage groups were:

Group	Dietary Level (ppm)	No. of Animals Male	Female
1	0	105	105
2	1	70	70
3	20	70	70
4	400	105	105
5	400 of Wessalon S	70	70

"The first 60 male and female mice in each group made up the main experimental groups which were used primarily for carcinogenic evaluation. They were not used for provision of blood samples except at the termination of the study or due to absolute necessity. The remaining animals in each group made up the satellite groups which were used for provision of blood samples and were not included in the carcinogenic evaluation. These mice were discarded after blood sampling at week 82.

"The animals were maintained in air-conditioned quarters with food and water available ad libitum and were housed five animals per cage in polypropylene cages providing 455 sq cm of floor area (Isocage-Laboratory Products, Inc., No. 18052). The diet was Wayne Lab-Blox Mash.

"Dietary mixtures were prepared as required in a Hobart laboratory mixer, and assayed immediately to assure that the desired concentration limits for each group were not exceeded.

"Blood samples at 0, 2, 4, 5, 8, 12, 13, 25, 26, 42, 45, 50, 51, 52, 79, and 80 weeks were obtained from the orbital sinus of anesthetized (halothane) satellite animals and the animals were returned to the colony. Blood samples at week 82 were obtained from the posterior vena cava of anesthetized (pentobarbital sodium, 30 mg/kg, i.p.) animals by means of a 21 gauge needle and a 5 ml syringe." (p. 3)

2. Parameters Examined

Toxic signs and symptoms, body weight, food and water consumption, ophthalmoscopy, hematology, blood chemistry, urinalysis, cholinesterase (erythrocyte, plasma, and brain), distribution of mortality, organ weights, gross necropsy, histopathology.

II. RESULTS

A. Toxic Signs

According to the study authors' comments, no dose-related toxic signs were observed. However, the report advises that males were observed to be unusually combative. As described in the report, all groups and, hence, presumably the controls, were included in this observation.

Combativeness is a parameter that should have been more fully characterized. It would be important to know whether combativeness was dose related.

Although many control and experimental animals died prior to completion of the study, the distribution of mortality was not suggestive of any compound exposure-related toxicity.

B. Body Weight

There were no remarkable effects of the compound on body weight during the exposure period.

C. Food and Water Consumption

No remarkable effects of the compound under study were observed on food consumption with the exception that females receiving 400 ppm NTN 8629 consumed less food than females receiving the control diet in 41 of the 82 weekly intervals. A summary table (p. 11) on food consumption also incorporates NTN 8629 consumption data. On a body weight basis, female mice consumed approximately 18.3, 22.4, and 10.5 percent more compound than did male mice when fed 1, 20, and 400 ppm diets, respectively. This may explain the effects on food consumption of females in the high-dose group as disclosed above.

The study authors' assert that water intake was unaffected (unable to locate any water consumption data, however).

D. Ophthalmoscopy

Ophthalmic examinations performed on all mice in the groups receiving the control diet and the 400 ppm NTN 8629 diet revealed opacities in a few mice of the high-dose group, but none in the control group. Specifically, three males and six females were observed to have opacities in one or both eyes. (No data presented).

It is unfortunate such observations were not made on the 1 and 20 ppm treated and Wessalon carrier groups. There appears to be no table of data covering these observations. This constitutes a deficiency.

E. Hematology

A variety of hematologic tests were reported. Hemoglobin, packed cell volume, red blood cell count, and white blood cell count were not affected in the 400 ppm

group in any consistent time-related manner for periods of exposure up to 80 weeks. Higher mean values for RBC were reported at the 51-week time interval for both control and 400 ppm NTN 8629 treated groups.

F. Blood Chemistry

A variety of blood chemistry parameters were evaluated at periodic intervals ranging up to 82 weeks of study. These are listed in the study report, page 5.

There were no remarkable compound-related effects on the various parameters with the possible exception of the following: blood glucose was significantly elevated ($p < 0.05$) in both males and females after 82 weeks of feeding the 400 ppm NTN 8629 diet. Also, sodium was significantly lowered in male but not in female mice at the 400 ppm level. On the other hand, cholesterol was significantly elevated in the treated females at this same dose.

G. Urinalysis

Administration of the test compound at 400 ppm in the diet over the period of 78 weeks did not result in any meaningful changes in the parameters examined.

H. Cholinesterase

The following indicates the degree of inhibition of the various cholinesterase as seen for the various dose at various time interval of exposure.

Cholinesterase Inhibition, %

Time	Dose	Plasma		Erythrocyte		Brain	
		M	F	M	F	M	F
26 weeks:	1 ppm	2	12 (13 weeks = 0) (50 weeks = 0)	45 (13 weeks = 0) (50 weeks = 0)	14 (13 weeks = 0) (50 weeks = 0)		
	20 ppm	88	--	25	62		
	400 ppm	86	--	36	--		
79 weeks:	1 ppm	0	0	0	0		
	20 ppm	82	73	23	2		
	400 ppm	98	98	41	25		
83 weeks:	1 ppm					0	0
	20 ppm					0	11 (not significant)
	400 ppm					45	62

From the above table, it is concluded that the NOEL for the brain enzyme is 20 ppm for both sexes. The LOEL for plasma and erythrocyte cholinesterase is 20 ppm or less. Due to lack of zero time activities for the two enzymes for treated groups and ambigious inhibitions at 1 ppm (e.g., 26 weeks) a definitive statement is not possible for the latter two enzymes at the 1 ppm dose.

I. Distribution of Mortality

The distribution of mortality is tabulated on page 8 of the study. While mortality is generally higher in males than females, there does not appear to be a dose-related increase in mortality for either sex. It should be noted, however, that loss of animals in all groups was excessive and may have obscured the finding of a positive dose-related effect on mortality.

J. Organ Weights

A review of tabulated data (pp. 95-96) on mean absolute and relative organ weights (heart, liver, spleen, kidneys, adrenals, gonads, brain, pituitary, and thyroid) of treated animals versus controls were unremarkable.

The number of animals for which organ weights were determined is indicated below:

Male	19 (control), 25 (1 ppm), 15 (20 ppm), 17 (400 ppm), 21 (carrier)
Female	28 (control), 29 (1 ppm), 25 (20 ppm), 28 (400 ppm), 29 (carrier)

These numbers correspond reasonably well with the number of mice surviving at the time of sacrifice, as shown in the tabulation of the Distribution of Mortality.

K. Gross Necropsy and Histopathologic Examination

Though some histopathologic data are reported for many of the animals, the study is severely compromised by a deficiency of histopathologic data as a consequence of early deaths and autolysis of animals in all study groups. The following tabulation provides an estimate of such loss:

		A	%	B	%
Group I	M	41	68	39	65
	F	34	57	30	50
Group II	M	33	55	32	53
	F	29	48	23	38
Group III	M	45	75	33	55
	F	40	67	36	60
Group IV	M	43	72	47	78
	F	32	53	33	55

Where: Column A is the number of mice dead prior to terminal sacrifice.

Column B is the total number of mice with markedly inadequate histopathologic data due primarily to autolysis.

The percent of animals essentially lost with respect to providing reliable histopathologic data is substantially in excess of the 10 percent allowed by guidelines.

Individual autopsy and histopathology sheets for each mouse examined in the study have been reviewed. Listed below is a tabulation of the number of mice in each group examined histopathologically.

Mice Examined Histopathologically

Dose Group / Sex	Control M	Control F	1 ppm M	1 ppm F	20 ppm M	20 ppm F	400 ppm M	400 ppm F
Table I (No page number provided)	47	54	54	53	57	57	42	53
Table I (pp. 124- 128)	45	53	55	53	56	54	41	53
Table III (EPA Reviewer findings)	47	54*	55	53	56	56**	41	53

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* Rat #127 - Data only on the heart. If not counted, figure reduces to 53.

** Rats #421, 422 (the first two in this group) are actually reported as male on histopathology sheets and male organs appear for these in Results. If deleted, figure reduces to 54.

Note: The study groups contained 60 animals each.

Tumor Incidence:

The following table summarizes the number and anatomic site of tumors identified:

	Males				Females			
Dose Level - PPM	0	1	20	400	0	1	20	400
Adrenal: Cortical Adenoma				1				
Anorectal Collision Tumor		1						
Ceacum: Reticular Cell Sarcoma					1			
Fibrosarcoma (Location not clear)						1		
Hemangioendothelioma							1	
Kidney: Reticular Cell Sarcoma					1		1	1
Liver: Adenoma			2					
Cholangiocarcinoma				1				
Hemangioma			1					
Hepatacarcinoma			1					
Hepatacellular Carcinoma	2	1		1				
Hepatoma		1	1	4				3
Reticular Cell Sarcoma						1	1	
Lung: Adenoma	1	5	2	4	1	4	1	2
Adenocarcinoma	2							
Beginning Malignant Transformation						1		
Papillary Acinar Carcinoma							1	
Reticulum Cell Sarcoma			1					
Lymphatic: Reticulum Cell Sarcoma			1					
Mammary Gland: Adenocarcinoma		1		1			1	
Mammary Gland and Skin: Cystadenocarcinoma							1	
Myxoma: (unknown site of origin)			1					
Ovary: Adenoma						1		
Reticulum Cell Sarcoma								1
Pancreas: Hamartoma					1			
Peripancreatic Lymph Node: Reticulum Cell Sarcoma						1	1	
Perisalivary Lymph Node: Reticulum Cell Sarcoma					1			
Skeletal Muscle: Giant Cell Fibrosarcoma								1
Reticulum Cell Sarcoma				1				
Spindle Cell Sarcoma		1						
Skin: Fibrosarcoma	1							
Hyperkeratotic Epithelioma			1					
Spleen: Hemangioma	1							
Subcutaneous Tumor: Adenocarcinoma	1							
Submaxillary Gland: Salivary Duct Adenoma			1					
TOTAL	8	10	12	13	5	9	8	8

Conclusions:

1. The evaluation of oncogenicity is severely compromised by the extensive autolysis observed in all groups. Such loss far exceeds the figure of 10 percent recommended by the guidelines, and renders the study invalid.

2. The technical grade of NTN 8629 used, described as 93.2 percent pure, was provided by Bayer, AG.
A complete analysis of the product to include impurities, etc., is required.

Additional conclusions as formulated by the study authors' and which we share follow:

A significant decrease in food consumption was observed, after 14 weeks, in female mice fed 400 ppm NTN 8629 but not in those fed 20 ppm.

Food intake in male mice fed a diet containing 400 ppm NTN 8629 was not affected.

Mortality in male and female mice was not affected by NTN 8629 fed in the diet at a concentration of 400 ppm for 80 weeks.

Significant decreases in plasma and red blood cell cholinesterase activities were observed in male and female mice receiving $\geq$ 20 ppm NTN 8629 in the diet.

RCB cholinsterase LOEL = 20 ppm or less
Plasma cholinsterase LOEL = 20 ppm or less
Brain cholinsterase NOEL = 20 ppm

Core Classification: Invalid

JOB:92859:Little:9/25/85:KENCO:DAA
REVISED:86745:Little:12/30/85:EK

Study: Evaluation of the Teratogenic Effect of NTN 8629 in the Rabbit

Laboratory: Institute of Comparative and Human Toxicology, Albany Medical College, Albany, NY and International Center of Environmental Safety, Albany Medical College, Holloman AFB, New Mexico.

Study Number and Date: Tab 23, October 6, 1977

Accession Number: 073093

Material Tested: NTN 8629 (94.6% Purity, Bayer, AG)

Animals: Dutch Belted Rabbits

Fifty female dutch belted rabbits were divided into groups of 10 pregnant rabbits each and administered prothiophos in sesame seed oil via stomach tube on days 7 thru 16 of gestation. Animals in groups I through V received the following daily doses, respectively: vehicle controls, 0.15 mg/kg, 1.5 mg/kg, 15 mg/kg, and 50 mg/kg. Animals were weighed daily during the dosing period, and observed for signs of toxicity. Animals were sacrificed at day 29 of gestation and the uterus examined in situ. According to the study authors, the number and location of implantation sites were first recorded and then the intact uterus was excised to determine the number and location of live fetuses, dead fetuses, and resorptions. Each fetus was examined for any gross external abnormality. The fetuses were weighed, and 1/3 of the litter fixed in absolute ethanol for subsequent skeletal examination. The remaining fetuses were fixed in Bouins Fixative for subsequent visceral examination.

There were no spontaneous deaths of pregnant rabbits in any of the groups under study. The percentage of viable young was greater than 90 percent in groups I through IV. In group V, the figure was 77.5 percent. The number of dead and resorbed fetuses was 4 to 5 in each of groups I through IV, but was 18 in group V. The increase in the number of dead and resorbed fetuses in Group V is attributable to excesses in three animals: #901, #904, and #930. It is also to be noted that in #930, all nine implants were located on the right side and five of these were dead. The study authors suggest that the large number of implantation sites on one side of the uterus in rat #930 could account for the losses from that animal, but offer no explanation for the losses observed in the other two animals. Nonetheless, these losses are considered compound related.

Average fetal weights of dosed animals were not significantly different from the control value. Also, there were no gross abnormalities of the skeleton identified on fetal examination.

This study was very limited in scope as evidenced by the lack of data showing individual animal macro- or microscopic examinations. The authors merely state that fetuses were counted, weighed, and examined for visceral and skeletal abnormalities. External examination of fetuses, gross evaluation of the skeleton, and dissections of abdominal, thoracic, and cranial cavities were all simply reported as disclosing no compound-related abnormalities.

1. There was no evidence of toxicity in the pregnant females. It would have been appropriate to have determined cholinesterase activity. If such data exists, it should be submitted.

2. Data presented included counts of implantation sites; live, dead, and resorbed fetuses, and total litter and individual fetal weights. There was an increase in resorptions and fetal deaths in the 50 mg/kg group which is considered compound-related and an index of "Developmental Toxicity."

3. There was no effect on fetal weight in any of the groups receiving NTN 8629.

4. An analysis of the sample of NTN 8629 employed in this study was not provided, and must be submitted.

5. This reviewer is left with doubts as to the thoroughness of the gross observations and is concerned about the lack of microscopic examinations of tissues. Accordingly, no conclusion can be reached concerning the teratogenic potential of the test material.

Individual animal and litter data are required. Until such data are received and evaluated, this study is classified as Core Supplementary.

Study: Administration to Rat During Organogenesis Period

Laboratory: Institute for Animal Reproduction
Fukaya 1103, Dejima-Mura, Miiharu-Gun Ibaraki-Ken

Study Number and Date: Tab 19, July 21, 1977

Accession Number: 073098

Material Tested: NTN 8629 (Developed by Nihon Tokushu Noyaku Seizo Co., Ltd.; Purity not specified).

Animals: Wistar Imamichi SPF Rats

Method:

Wistar-Imamichi strain SPF rats, 30/group, were administered doses of 0, 0.8, 4.0, and 20.0 mg/kg daily by subcutaneous injection (dorsal neck region) during the 7th to 17th day of pregnancy. On the 20th day of pregnancy, 2/3 of the dams from each group were sacrificed and autopsied, while fetuses obtained from these were examined for teratogenic effects. The remaining 1/3 dams in each group were allowed to deliver for the purposes of studying the growth, differentiation, and reproduction of the F1 generation.

Principal findings are enumerated as follows:

A. F0 Generation

1. Symptoms of toxicity were apparent in the 20 mg/kg dosed pregnant dams. These included salivation, pilo-erection, and incontinence of urine. These symptoms did not disappear even after termination of dosing. Such symptoms were not observed in the lower dosed groups. In prelimary studies, the 20 mg/kg dose administered subcutaneously was found to remarkably inhibit serum and brain cholinesterase (data not shown).

2. Dams exposed at the high-dose level exhibited significantly reduced weight gain at days 14 and 20 of pregnancy. Weight gain was not adversely affected at the lower doses (table 1). High dosed animals also consumed less food and water (tables 5 and 6).

3. Dams in the high-dose group were described by the study author as having developed "neurosis," such that those animals exhibited disturbances at delivery and aberrant nursing behavior. Premature deaths of pups (table 8) born in that group were attributed by

the study authors to this compromised dam nursing behavior. Premature pup deaths were not observed in the other groups. Pup weights in the high-dose group were decreased relative to controls. The decrease was statistically significant for females.

4. The duration of pregnancy was significantly reduced in the high-dose group (table 8).

5. There were no differences in copora lutea, implants, and nonviable implants/litter for dams of any dose group compared to control values (table 8).

6. Dams from the high-dose group at autopsy on the 20th day of pregnancy exhibited increased adrenal gland weights and marked atrophy of the spleen and thymus. Liver and ovary weights were also reduced in the high-dose. There were no remarkable differences observed at autopsy in dams from other dose groups.

7. Parental (F0) organ weights among dams allowed to deliver were unremarkable in the 0.8 and 4 mg/kg groups. There are no data on the high dose group (table 7).

B. F1 Generation

1. Fetuses from the 20 mg/kg dose group of F0 dams weighed significantly less than control fetuses. Fetal weights in other dose groups were not affected (table 2). There were 18 cases of dwarfism among the 251 females examined in the high-dose group. Dwarfs were not observed in the other groups (table 2).

2. Among fetuses examined for skeletal defects the only major malformation was that of tailessness observed in the low-dose group. It should be noted that while abnormalities and variations were few and not dose related, all findings were in dosed groups, i.e., none were in controls (table 9).

3. There were a number of significant skeletal ossification defects in the high-dose group: 1) increased number of fetuses with poorly ossified regions of the skull, 2) increased number of fetuses with poorly ossified or unossified sternum, 3) decreased number ossified cervical vertebra and 4) decreased number of 1st manuphalanges ossified centers. There were no significant findings at the lower doses (table 10).

4. Visceral examination of fetuses were unremarkable with the exception of an apparent increased occurrence of dilated renal pelvis and hydroureter in the 4 and 20 mg/kg dose groups. These numerical increases (table 11) are not accompanied by statistical analyses, but appear to be statistically significant and dose related. There also appear to be dose-related increases in the number of fetuses with subcutaneous edema (table 11).

5. Among F1 pups delivered, there were significantly less surviving pups in the 0.8 and 20 mg/kg dose groups as compared to control groups. There were no deaths among delivered pups in the control and 0.8 mg/kg dose groups; however, there were two in the 4 mg/kg and 6 in the 20 mg/kg dose groups. Sex ratios among pups were not affected at any dose. Perhaps the most conspicuous observation was the total loss of pups by the third day following delivery in the high-dose group. Pups in the low-dose groups exhibit significantly greater survival than controls (table 8).

6. Pup weights prior to weaning were increased somewhat in the low-dose group, but no adverse effect was noted in the 4 mg/kg group and no data exists on the high-dose group as a consequence of the total mortality of the group (table 12).

7. Additional examinations of pups from the 0, 0.8, and 4 mg/kg but not 20 mg/kg dose groups with respect to morphological differentiation (table 13), body weight after weaning (tables 14 and 15), mean food and water intake of males (tables 16 and 17), and organ weights of male and females (spleen, thymus, adrenal included among others) (tables 19 and 20) did not reveal unusual findings <u>except</u>: mean food and water intake for males was numerically down in the 4 mg/kg dose group, but no statistics are included in the reported finding (tables 16 and 17). Consistent with this was an apparent increase in the efficiency of food utilization for males in the 4 mg/kg dose group (table 18).

   Female body weights and reproductive function (table 21) of the F1 generation revealed nothing unusual for the 0.8 and 4 mg/kg dose groups. Likewise food and water intake levels and food efficiency were not remarkably different in these groups (tables 22, 23, and 24).

8. Results of Autopsy of F1 Dams on the 14th Day of Pregnancy.

Examinations of five dams from each dose group (0, 0.8, 4.0 mg/kg) revealed small but statistically insignificant declines in number of implants, number of corpora lutea, and live embryos among dosed groups. Other aspects of the autopsy were unremarkable (table 25).

9. Reproduction parameters for F1 generation dams were essentially unaltered in the 0.8 and 4 mg/kg dose groups (table 26).

Discussion

A variety of symptoms of maternal toxicity were evident in the high-dose group dams. These symptoms including salivation, piloerection, reduction in weight gain, and incontinence of urine, probably were the result of cholinesterase inhibition, an opinion reinforced by the actual finding of serum and brain cholinesterase inhibition in pilot controls. However, in addition to these signs of maternal toxicity, dams in the high-dose group were described in the report as exhibiting abnormal behavior following delivery as evidenced by a suppressed nursing instinct and reduced propensity to dispose of placentas at birth of litters. The study directors appear to ascribe this behavioral effect to CNS cholinesterase inhibition. While this behavioral effect certainly is a possible consequence of cholinesterase inhibition, the compound may elicit abnormal neurological effects by other mechanisms.

There are published works on organophosphates containing various substituted phenols which, by structural analogy, notably the presence of the 2,4-dichlorophenol moiety, indicate a potential for prothiophos to be neurotoxic. [Aldridge and Barnes (1967); Aldrige and Barnes (1960); Lomis (1978); Aldridge, et. al. (1969).] Those considerations reinforce the importance of a neurotoxicity study on this compound.

The complete mortality of F1 pups in the high-dose group was attributed by the study directors to the abnormal nursing behavior of the dams. This may be the correct explanation for the pup deaths, but information in the report is not sufficiently detailed to rule out the possibility of in utero toxicity manifested in pup death following delivery. In retrospect, perhaps some litters from high-dose dams could have been placed with control lactating dams to see if proper feeding and survival of pups was possible. This would help resolve the question of whether this finding of total pup death in the high-dose group was one of maternal or fetotoxicity.

Among dams autopsied on the 20th day of pregnancy, those of the high-dose group exhibited increased adrenal gland weights and

marked reductions in spleen and thymus gland weights. Also, there was a reduction of liver weight. The study directors do not adequately address the implications of these findings. Necrotizing effects on the spleen and thymus indicate possible detriment to immunologic capability.

It should be noted that prothiophos contains a 2,4-dichlorophenol substitute (moiety), and that a number of chemically similar chlorinated hydrocarbons have been clearly shown to induce immunosuppression or reduced host resistance to infectious agents. Furthermore the issue is complicated by the finding of an extreme immunosuppression induced by tetrachlorodibenzo-p-dioxin (TCDD) in many species and the likely contamination of chlorophenol derivatives by TCDD. TCDD has been shown to suppress immune responses in offspring of pregnant or nursing rats and mice. Furthermore, atrophy of the thymus and decreases in cortical thymocytes is a very sensitive index of TCDD exposures. [Faith and Moore (1977); Gupta et al. (1973).] After oral dosing, TCDD concentrates mainly in the liver and fat of the rat, but is also found to a lesser extent in the thymus, spleen, and kidney. TCDD is known to produce a pronounced atrophy of thymus, spleen, and peripheral lymph nodes of rats, mice, and guinea pigs [Moore and Faith (1976)]. In guinea pigs, doses of TCDD as low as 0.04 μg/kg body weight weekly for 8 weeks yield statistically significant reductions in thymus weight [Vos, et al., (1973)]. Thus a scientific rationale does exist as a possible explanation for the observed effects of prothiophos on the weight changes of the thymus, spleen, liver, and body weight of animals exposed in this study. Therefore, a thorough analysis of NTN 8629, including characterization of contaminants, is required.

Among fetuses autopsied from dams in the high-dose group, reduced fetal weight and several cases of dwarfism were found. Organ weights were not obtained. The finding of dwarfism is difficult to interpret. This may be a teratogenic or fetotoxic phenomenon consequent to exposure to prothiophos or its contaminants, and, hence, merits comment. Along these lines, Faith and Moore (1977) indicate that offspring of female rats dosed with TCDD pre- and postnatally show significant inhibition of growth as assessed by body weight measurements throughout the course of their experiment (145 days). Furthermore, as cited by these investigators, animals exhibiting decreased growth rates are described as resembling animals with "runt disease" or "wasting syndrome" induced by neonatal thymectomy (Parrott, 1962; Azar, 1964), neonatal treatment with corticosteriods (Schlesinger and Mark, 1964; Winick and Coscia, 1968), or neonatal treatment with allogenic lymphocytes (Kaplan and Rosston, 1959; Billingham and Brent, 1959). All of the aforementioned treatments produce thymic dysfunction, as does TCDD. TCDD profoundly affects the thymus, causing loss of thymic cortical tissue and normal thymic architecture (Gupta, et al., 1973; Vos, et al., 1973).

Given uncertainty as to whether the differences between the terms "dwarf" and "runt" as employed in the works under discussion is distinct or one of semantics, it was considered relevant to mention the runting effect of TCDD. It is quite possible that dwarfism as observed in this NTN 8629 study is clearly different from runting and has a different etiology. However, in another way runting and dwarfism may both be effects of TCDD exposure, the former due to the compound's peculiar effects on the thymus and the latter to other teratogenic mechanisms. There also remains the possibility of a teratogenic or fetotoxic effect of prothiophos itself, or its metabolites.

In summarizing these considerations, dwarfism and reduced fetal weights observed in the high-dose group are consistent with TCDD or perhaps other chlorinated hydrocarbon exposure which would adversely affect the thymus (and other aspects of the immune system). The complete mortality within 3 days of birth among pups of the high dosed dams allowed to deliver (table 8) may just as well have been the consequence of compromised immune capability as to the maternal neglect claimed by the study director (pp. 2, 14, 19, 21). Since dams and pups from the lower dose groups were not obviously affected, the NOEL for this toxic response appears to be > 4 mg/kg < 20 mg/kg. In retrospect, a histopathologic examination of the thymus gland (particularly) of offspring at all dose levels would have been appropriate in identifying a NOEL.

Fetal examinations do suggest a teratogenic response. Where skeletal malformations were concerned, there was one tailless animal in the low-dose group and several other abnormalities and variations all within the dosed groups only, but not manifest in a dose-response manner (table 9). There were a significant number of skeletal ossification abnormalities in the high-dose group which included an increased number of fetuses with poorly ossified regions of the skull, increased number of fetuses with poorly ossified or unossified sternum, a decreased number of ossified cervical vertebrae, and a decreased number of 1st manuphalange ossified centers (table 10). Visceral examinations of fetuses revealed increased incidences of dilated renal pelvis and hydroureter in the 4 and 20 mg/kg dose groups (table 11).

Viewed in its entirety, this study does indicate that prothiophos itself, a metabolite, or contaminant manifests a teratogenic or fetotoxic, and possibly a behavioral effect in rats. Furthermore, these effects may be mediated via effects on the immune system. TCDD, a highly suspect contaminant of prothiophos is a well-recognized teratogen (Harbison, 1980).

Summary of Principal Findings

1. Symptoms of cholinesterase inhibition in dams of the high-dose (20 mg/kg) group were observed. Serum and brain

cholinesterae inhibition were observed in pilot studies at the dose.

2. An adverse behavioral effect (nursing behavior) was apparent among high-dose dams. This could be a manifestation of neurotoxicity of prothiophos. As discussed, prothiophos bears structural similarity to known neurotoxic agents cited. This finding underscores the need for a neurotox study on prothiophos.

3. Marked atrophy of the spleen and thymus were observed among high-dose dams.

4. Dwarfism and reduced fetal weight in general were observed among fetuses of high-dose dams.

5. Increases in dilated renal pelvis and hydroureter were observed among pups in the 4 and 20 mg/kg dose groups. Such effects generally are considered to be reversible during postnatal development; and, hence, are to be viewed as manifestations of "developmental toxicity" as opposed to teratogenic effects. Teratogenic effects are irreversible in nature. Therefore, with respect to these findings of developmental toxicity, the NOEL = 0.8 mg/kg.

6. A number of defects in skeletal ossification were observed among pups in the high-dose group. Such effects generally are reversible during postnatal development and are to be viewed as evidence of "development toxicity" as opposed to teratogenicity of the test substance. In this case, NOEL = 4 mg/kg.

7. Therefore, there was no evidence of teratogenicity observed in this study at doses up to and including 20 mg/kg.

Deviations From Guidelines:

1. Compound was administered by subcutaneous injection in the dorsal neck region and is considered inadequate for evaluation of dietary exposure to residues in food.

2. No historical control data apparently have been submitted on the Wistar-Imamichi strain SPF rat employed in this study by this method of application of test substance.

3. No information has been provided on the purity or chemical analysis of NTN 8629 (Nihon Tokushu Noyaka Seizo, Co., Ltd.) employed in this study. Most significant would be an analysis for TCDD.

Core Classification: Supplementary

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Study: Insecticide NTN 8629 - Administration to Pregnant Rabbits During Organogenesis Period

Laboratory: Institute for Animal Reproduction
Fukaya 1103, Dejima-Mura, Niiharu-Gun Ibaraki-Ken.

Study Number and Date: Tab 21, July 21, 1977

Accession Number: 073098

Material Tested: NTN 8629 (purity not provided)

Animals: New Zealand White Rabbit

Forty pregnant New Zealand White rabbits (10 rabbits per group) were administered NTN 8629 subcutaneously at doses of 0, 8, 16, and 32 mg/kg from the 6th through the 18th day of pregnancy. The compound under study was, for purposes of subcutaneous injection, emulsified in 1% Tween 80.

The doses selected were based on pilot studies (route of administration not provided) showing that doses up to and including 20 mg/kg did not exert toxic symptoms excepting slight brain cholinsterase inhibition at the 20 mg/kg dose. Dams were sacrificed on the 28th day of pregnancy and both the dams and fetuses were examined for defects.

Examinations consisted of the following:

1. General clinical symptoms of dams during pregnancy, abortions, and premature births;

2. Weight gain and food intake of dams;

3. Autopsy of dams to count corpora lutea, implants, viable and dead fetuses, and resorptions;

4. Examination of fetuses including external malformation, and weight. Approximately 1/2 of the fetuses were used for skeletal examination and the remainder for visceral examination.

a. Examination of Dams

No deaths occurred nor were there toxic symptoms or clinical findings at any dose level. Two dams, one each from the 16 and 32 mg/kg dose groups, completely terminated pregnancy via resorption of all embryos, 8 in each case. In addition, one dam from each of the high dose groups resorbed five of seven embryos/fetuses.

During the period of pregnancy, dosed animals showed no statistically significant differences in weight gain or in food intake from the control group.

b. Examination of Fetuses

At autopsy, the dosed groups did not differ significantly from the controls for number of corpora lutea, implants, viable fetuses, or in mean fetal weight. Though there was no difference between groups in the implantation index (implants/corpora lutea), there were statistically significant increases in embryo/fetal losses in the two high dosed groups compared to controls. (Appendices 2-1 through 2-4; table 3).

Only one major malformation, horoacranius accompanied with manus valga, was observed. This occurred in the high-dose group. In addition to this major malformation, there were other abnormalities as follows: a) one case of hydroureter in the 8 mg/kg dose group; b) one finding of an abnormal arterial path (transposition of the right subclavian artery) in the 8 mg/kg dose group; c) in the skeletal examination, there was one cranial anomaly (partial separation of the parietal bone) observed in the 16 mg/kg dose group; d) one bilaterally shortened first rib was detected in each of the 16 and 32 mg/kg dose groups; and e) a statistically significant increase in retarded ossification of caudal vertebrae in the 16 mg/kg dose group.

Discussion:

The dose levels selected in this study were based on pilot studies showing that doses up to and including 20 mg/kg administered subcutaneously caused slight brain cholinesterase inhibition at the 20 mg/kg dose. Cholinesterase assays were not reported in the final study, but dose levels employed were within the demonstrated effective dose range.

A major abnormality was observed in the high-dose group as well as excess resorptions in the middle- and high-dose groups. The study authors hold the view that the major malformation observed in the high-dose group was of a spontaneous nature, unrelated to compound administration, however, this cannot be conducted from the data presented.

Malformations do occur with measurable frequencies. For instance, Palmer (1978) indicated the frequency of spontaneous major malformations, minor visceral anomalies, and minor skeletal anomalies in New Zealand White rabbits occur at rates of 0.74, 2.53, and 8.60 percent, respectively. Applying the 0.74 percent figure for major malformations to the entire study cohort of viable fetuses would indicate 1.86, or approximately two, likely spontaneous malformations in the major category. Therefore, the

finding of one such fetus in this cohort is not surprising. However, the fact that the major malformation is extremely severe (horoacranius - without a head) and rare, and occurred in the high-dose group only enhance our concern.

That there may be a teratogenic effect associated with the administration of the compound is exacerbated by the finding of excessive resorptions in the high- and middle-dose groups. This phenomenon is characteristic though not uniquely so of teratogenesis (Harbison, 1980). With respect to this latter finding, the study authors make the statement that: "After administration, a dam from the 16 mg/kg group and another dam from the 32 mg/kg group discontinued their pregnancies by the resorption of all embryos, which had presumably occurred at a rather early stage of pregnancy. However, the other members of both groups showed no difference from the control animals in the counts per litter of dead and resorbed embryos and fetuses" (p. 6). This latter claim cannot be fully substantiated. It is true that the death rates for embryos/fetuses are reported in table 3 of the study to be 5.5, 4.2, 29.7, and 22.5% for the 0, 8, 16, and 32 mg/kg dose groups, respectively, where the rates for the two higher dosed groups were very significantly different from that of the control. However, in each of the two high-dose groups there was an additional dam which resorbed 5/7 embryos/fetuses. Thus, in each of the two high-dose groups there were two dams which resorbed large fractions of their conceptuses. In commenting on "discontinued pregnancy" the study authors indicate (p. 6) that this "could hardly be caused by the lethal effect of the administered compound on embryos and fetuses, and it seems more reasonable to assume that the compound had some effect on the supporting mechanism of pregnancy" or could have been spontaneous in nature. This reviewer is not sure what "effect on supporting mechanism of pregnancy" means unless maternal toxicity is envisioned. In any case, to be convincing, a more plausible scientific explanation is necessary.

It is possible that maternal toxicity resulted from cholinesterase inhibition although this was not evident from the data presented.

While resorptions observed in the middle- and high-dose groups could have been spontaneous in nature, the extensive resorptions in the high- and middle-dose groups are considered due to fetotoxic or teratogenic effects of the compound.

Conclusions:

1. Significant deviations from EPA guidelines include:

   a. Guidelines suggest that when rabbits are employed in teratogenesis studies, 12 pregnant animals/dose be employed. In the current study, 10/dose were used.

   b. Guidelines indicate that the highest dosage level should induce some overt maternal toxicity such as slight weight loss, but not more than 1 percent maternal deaths. In the study in question there were no signs of maternal toxicity (possibly excepting resorptions) or deaths at the highest dose. Cholinesterase was not assayed except in the pilot study where 20 mg/kg, the highest dose evaluated, reportedly inhibited brain cholinesterase, slightly. It would not be appropriate for one to assume, in the absence of data, that the 32 mg/kg dose inhibited cholinesterase.

   c. According to guidelines, the test substance is usually administered orally unless characteristics of the compound or pattern of human exposure suggest otherwise. The test substance in this study was administered by subcutaneous injection and is considered inappropriate for evaluating the hazard from ingesting residues in food.

   d. Dams, when sacrificed, should be examined macroscopically for any structural abnormality or pathological changes which may have influenced the pregnancy. There are no data addressing this aspect.

   e. Guidelines emphasize the necessity to consider the historical teratogenicity data on the species/strain tested. Historical control data on the rabbit used in this study was not presented. This is of importance in view of the case of horoacranius.

2. A NOEL for teratogenicity, fetotoxicity, or maternal toxicity was not clearly demonstrated. The study should be repeated in accordance with acceptable guidelines for such studies.

3. Data are classified Core Invaild and cannot be upgraded because of the method of dosing and number of animals used.

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Study: Evaluation of the Reproductive and Teratogenic Effects of NTN 8629 in the Rat

Laboratory: Institute of Comparative and Human Toxicology, Albany Medical College, Albany, NY and International Center of Environmental Safety, Albany Medical College, Holloman AFB, New Mexico.

Study Number and Date: Tab 22, October 6, 1977

Accession Number: 073093

Material Tested: NTN 8629 (94.6%, Bayer, AG)

Animals: Long-Evans Rat (adult, virgins)

This is a three-component study designed to address the effects of NTN 8629 at three dose levels on:

1. Implantation during days 0 to 7 of gestation.
2. Organogenesis during days 7 to 16 gestation, and
3. Fetal growth and development during days 16 to 21 of gestation.

Methods:

Female rats used in this study were impregnated through "successful mating." The method of confirmation of "successful mating" was not provided. NTN 8629 was administered in sesame seed oil by gavage. Dosages used in each of the three study groups were 0, 0.25, 2.5 and 25.0 mg/kg. These doses were administered daily during the periods of investigation (as indicated above for the three studies). In each of the three studies, ten animals per control or dose group were used.

Results:

1. Implantation (days 0 to 7 of gestation)

There were no spontaneous deaths or signs of toxicity in any of the pregnant rats evaluated in this experiment. There were no differences in the number of implantations, resorption sites or dead fetuses per rat in any dose group compared to controls. The results of this experiment indicate that the oral administration of NTN 8629 up to 25 mg/kg to pregnant rats from days 0 to 7 of gestation did not intefere with implantation.

2. Organogensis (days 7 to 16 of gestation)

There were no spontaneous deaths or signs of toxicity, no effect on the number of implantation sites and no dead fetuses in any of the litters examined. The number of resorbed fetuses

was the same in Groups I, II, and III. However, there was an increase in the number of resorptions in the Group IV rats. The increase in resorption was due to a total of 10 resorptions in one rat.

There was no evidence that NTN 8629 administered from days 7 to 16 of gestation at 25 mg/kg was embryotoxic or teratogenic.

3. Fetal Growth and Parturition (days 16 to 21 of gestation)

The number of viable young was equivalent in the control and lower dosed groups, however, the number of viable young was markedly reduced in the high-dose group and attributed to excess deaths occurring in four dams. Parturition was also delayed (about 2 days) in the high-dose group.

No gross external or skeletal abnormalities were observed at any dose.

Conclusions:

1. The oral administration of NTN 8629 to pregnant rats from days 0 to 7 of gestation did not intefer with implantation at doses up to 25 mg/kg.

2. When administered during the period of organogensis, days 7 to 16, there were no adverse effects noted in the low-and middle-dose groups with respect to number of dead fetuses or resorptions. However, there was an increase in the number of resorptions in the high-dose group, indicating a NOEL = 2.5 mg/kg for developmental toxicity. There were no visceral or skeletal abnormalities observed, and litter weights and fetal weights from dosed animals were not different from those of controls.

3. At the 25 mg/kg dose level administered from days 16 to 21 of gestation, NTN 8629 interfered with parturition. In 4 of 10 rats parturition occurred up to 36 hours later and over a longer time interval than was observed with control animals. In addition, there was an associated increase in the number of dead pups delivered at the high dose.

Comment:

The no-effect level for the overall study appears to be 2.5 mg/kg. However, the study was very limited in scope in that there are no data showing individual macro- or microscopic examination. In particular, there is no evidence that would establish whether NTN 8629 as administered had any effect on the weight or morphology of organs and there is no record of gross observations, only verbage to the effect that some were observed.

Analytical information addressing the complete composition of NTN 8629 is needed.

Individual animal and litter data are required. Until such data are submitted and reviewed, this study is classified as invalid and could be updated to supplementary by submitting gross and histopathologic data, and organ weight data.

Study Title: Administration to Rat Before and During the Early Stage of Pregnancy

Laboratory: Institute for Animal Reproduction. Fukaya 1103, Dejuma-Mura, Niiharu-Gun Ibaraki-Ken.

Date: Tab 18, July 21, 1977

Accession Number: 073098

Material Tested: NTN 8629, Developed by Nihon Tokushu Noyaku Seizo Co., Ltd.

Animals: Wistar Imamichi Rat (SPF)

The stated purpose of this study was to investigate the effects of NTN 8629 on the reproductive function of rats. The compound was administered at various doses to 6-week old male rats and 11-week-old female rats for 60 days and 14 days, respectively. Following the series of compound injections female rats were mated with males at the rate of 1 to 1 and the course of pregnancy followed.

Methods and Materials: (Quoted from page 2 to 3)

"1. Experiment animal: Eighty 6-week-old males and eighty 11-week-old females of Wistar-Imamichi rat (SPF) were reared in a barrior at room temperature 25 ± 1 °C and humidity 55 ± 5 percent during the experiment period. The rats were made accessible to the solid type feed for rats and mice made by Funabashi Farm, and water.

"2. Dosage and the number of rats for each group based on preliminary tests* conducted by the Institute for Animal Reproduction, dosages were set as follows:

Control Group: 0 mg/kg (solvent only, 1% Tween-80 solution)

Low Dosage: 2.0 mg/kg
Medium Dosage Group: 4.0 mg/kg
High Dosage Group: 8.0 mg/kg

Each group consists of 20 male and 20 female rats.

* Preliminary test results
By the same method as that of the present experiment, male and female rats were administered the compound in the 6 doses of 0.16, 0.8, 4.0, 20.0, and 60.0 mg/kg, and compared with the rats of Control Group as to poisoning symptom, body weight, mating index, fertility index, and external malformation of fetuses."

The remainder of the materials and methods section is paraphrased from the corresponding section of the study report (pp. 3 to 6).

NTN 8629 (of unspecified analytical purity) was emulsified in 1 percent Tween-80 and was admistered to females by subcutaneous injection of the upper back and to males by subcutaneous injection on the upper back and inguinal region alternately. A constant volume of administration of 0.25 ml/100 g body weight was employed. The compound was actually administered to male rats for more than 60 days from the start of the experiment (6-week-old) to mating. Females were administered the compound from the 14th day prior to mating (11-week-old) to the 7th day of pregnancy. (Note: This description differs somewhat from that of the stated purpose of the study).

The following observations were recorded:

1. General observations.
2. Body weight of males was determined at 6-day intervals and that of females once weekly.
3. Food and water intake and food efficiency were determined daily (apparently) for rats of both sexes prior to mating. During pregnancy, food and water intake were recorded during various periods of pregnancy, specifically 1 to 7 days, 8 to 14 days, and 15 to 21 days.
4. Observations were recorded with regard to determining or evaluating sexual maturity.
5. Animals were mated and observations were made to determine a) mating index (number of copulated males/number of used males) x 100 and b) the fertility index (number of pregnant females/number of copulated females) x 100. A statement follows as to what was done by males and females did not copulate but is not clear to this reviewer.
6. Pregnant (copulated) females were sacrificed by cervical dislocation on the 21st day of pregnancy and the following parameters evaluated.

    a. Number of corpora lutea.
    b. Number of implants.
    c. Number of viable and dead fetuses.
    d. Weight of viable fetuses.

    Macroscopic examination were conducted on all animals at autopsy. Weight of the following organs were determined: liver, kidney, spleen, heart, brain, hypopysis, thyroid glands, adrenal glands, thymus and gonads (for 10 male and 5 female rats, only)

7. All fetuses were examined for malformations. Half of the fetuses/litter were used for skeletal preparation for the examination of skeletal abnormalities. The other half of the fetuses/litter were dissected for evidence of visceral animals.

Results:

1. Results of the preliminary experiments involving dosages as high as 60 mg/kg are described as follows by the study authors, however no tables of data are presented in the results section for this trial run.

   "At 4 mg/kg no effects were observed on body weight, mating index on fertility index. At 20 mg/kg serious effects were observed. There were marked inhibition of male body weight gain and 1/5 of lots did not copulate, fertility index for females was only 25 percent of the normal. At the 60 mg/kg dose death was extensive during the 60-day period of compound administration." There were no comments or no effects seen at the lower doses of 0.16 and 0.8 mg/kg.

2. In the principal study. No symptoms of a general nature were noted through the rearing period. Male body weight during the 60-day period was depressed by NTN 8629 at 8 mg/kg beginning at the 12 week. Weight remained between normal for the remainder of the study. There was also a significant increase in body weight observed from the 24th to the 54th week (6 determinations) in the 2 mg/kg dose groups.

   There were no remarkable effects on female body weights during the fourteen days prior to pregnancy or the 21-day gestational period (tables 1, 2, pp 12 to 13).

3. Food and water intake and food efficiency data indicate that dosing with NTN 8629 had no significant adverse effect on males. There was a numerical reduction in all three parameters at the 7 to 12 day treatment point, perhaps reflecting a response to which the animals were subsequently able to adjust. No adverse effects were noted for females (tables 3 to 5, p. 14 to 16).

4. With regard to reproductive effects, the study authors indicate that male mating index was not affected at the 2.0 and 4.0 mg/kg doses, but apparently was decreased in the 8.0 mg/kg groups. The no-effect level for this parameter is 4.0 mg/kg. There were no adverse effects observed on the fertility index of females (appendices 4-1 through 4-4, pp. 32 through 35).

Where the question of success or lack of success in mating is concerned, the text is quoted as follows:

"To the male rats that did not copulate, that is, one from Control Group, 3 from 2.0 mg/kg Group, 2 from 4.0 mg/kg Group, and 7 from 8.0 mg/kg Group, opportunities for mating with untreated female rats as well as female rats of their own groups were given three times each, in other words, six times in all. As a result, those male rats succeeded in copulating except for two cases of 4.0 mg/kg Group and one case of 8.0 mg/kg Group. Among the copulated rats, however, only one untreated female rat each that copulated with a male rat of 2.0 mg/kg Group or that of 8.0 mg/kg Group became pregnant. There was observed a decline in copulation and fertilization among the male rats of 8.0 mg/kg Group.

On the other hand, in the later test on the female rats that did not copulate at the first mating, that is to say, one from 2.0 mg/kg Group, 3 from 4.0 mg/kg Group, and 7 from 8.0 mg/kg Group, the following results were obtained:

The cases in which the female rats that copulated with male rats of their own Groups or untreated male rats, were found at the rate of 1/1, 3/3, 1/2, and 3/7 for Control Group, 2.0 mg/kg Group, 4.0 mg/kg Group, and 8.0 mg/kg Group, respectively. Among the copulated female rats, however, there was one case of successful pregnancy (mating with an untreated male rat) in 4.0 mg/kg Group only. The rest female rats were all sterile. As seen from the abovementioned results, there was clearly noted an inhibition on copulating action in 8.0 mg/kg Group, as far as the test for reproductive function was concerned" (pp. 7, 8).

With regard to the above quotation, the following conclusions are evident:

a. Since 7 of 20 male - female pairs of the 8 mg/kg dose Group did not copulate in the initial pairing, as compared to 1/20 such "failures" for the control group, NTN 8629 apparently exerted an adverse effect on reproduction. Furthermore, of 7 untreated females subsequently mated with the 7 males of the 8 mg/kg dose group which did not successfully copulate with females of that same dose group, all but one pair copulated, yet only one of the seven untreated females became pregnant. This indicates a negative effect of NTN 8629 on male fertility at this dose. The study authors appear to acknowledge these adverse effects of NTN 8629 at 8 mg/kg on reproductive performance.

However, it should also be noted that when 3 males of the 2 mg/kg dose group male - female pairing which did not copulate were subsequently mated with 3 untreated females, only one female became pregnant, even though all three pairs copulated. This is indication of a negative effect of NTN 8629 on male fertility even at the low dose of 2 mg/kg.

b. When female rats which did not copulate with their respective male counterpart in the initial mating were mated with untreated males, only one female, that from the 4 mg/kg dose group, became pregnant. These findings demonstrate that at 2 mg/kg, NTN 8629 exerts an adverse effect on female fertility.

With respect to reproductive function impairment, a no-effect level cannot be identified in this study, as every level produced an adverse effect.

6. Table 6 p. 17 reveals that for those dams in each dose group which became pregnant, there were no adverse dose related findings observed with respect to: number of corpora lutea, implants, live and dead embryos or fetuses. An exception was a significant reduction in the total number of live fetuses in the 2 mg/kg dose group, i.e., 11.9 $\pm$ 3.5 vs 13.7 $\pm$ 2.2 for the control. This is another example of findings as in other studies wherein effects are seen at the low but not high doses.

7. One fetus from the 2 mg/kg dose group was characterized as having the following abnormalities: micrognathia, anophthalmia and tongue-tie. This defect occurred in a dosed group which experienced reproductive difficults as described above, in both male and female animals. Such possible adverse effects of NTN 8629 on reproduction make it difficult to accept the gross defect in this case as one of chance alone, unrelated to dosing. Exposure in this case occurred during the period of organogenesis.

8. Table 6 also shows that the implantation rate was significantly reduced in the high-dose, 8 mg/kg group, i.e., 76.7 percent, as opposed to 84.9 percent for the control.

9. Examination of fetuses at autopsy for abnormalities of visceral structures of the head, thorax and abdomen revealed none related to dosing. It may be noted that of tissues examined from the thorax, abnormalities of the thymus were observed in all dosed groups. None appeared in the control. However, the percentage of fetuses in each dose group exhibiting thymus defects was small, i.e. 3 percent, 1.7 percent, and 2.5 percent in the 2.0, 4.0, and 8.0 mg/kg dose groups, respectively. In the thorax, ventral ceptum defect was

increased in dosed groups with a dose response tendency. Also, one case of transposition of cardiac blood vessels was found in both the 2.0 mg/kg and 4 mg/kg dose groups (table 7, p. 18).

There were no remarkable findings with respect to skeletal abnormalities based on examinations of the skull, sternum, vertebral column, and appendages (tables 8 and 9, pp. 19 to 20).

10. In the absence of data we cannot determine if there were any macroscopic changes or abnormalities. The only organ changes revealed were the significant increase in spleen weight observed in males at all dose levels. In females, spleen weight was numerically increased in all dosed groups also, but significantly so only at the lowest dose, 2 mg/kg. It should be noted that thymus weight in males was numerically reduced in all dosed groups, but significantly so only in the low dose group, 2 mg/kg. Thymus weight from females was unaffected at any dose. Male body weight was significantly increased in the low dose group, but not altered at other doses. Female body weight was not altered in any group (tables 10 and 11, pp. 21 through 22).

Conclusions:

1. Male body weight NOEL = 4 mg/kg.

2. Female body weight NOEL = 8 mg/kg.

3. Male and female mating performance and fertility were impaired in the 8 mg/kg dose group. Male rats showed almost no interest in females at the time of mating (p. 10). The reason for this lack of interest is unknown, but is reminiscent of inhibited nursing behavior exhibited by females in another study (Administration to rat during organogenesis period, Tab. 19, Accession No. 073098).

4. Evidence suggests that fertility in both sexes was also impaired in the 2 mg/kg dose group. Hence, a NOEL for reproductive function cannot be identified.

5. Significant increases in spleen weight were found in male rats at all dose levels. In female animals increases in spleen weight were observed in all dose groups, but the increase was statistically significant only in the 2 mg/kg dose group.

6. Decreases in weight of the thymus gland were observed in male rats at all dose levels. These changes were statistically significant in the 2 mg/kg and 4 mg/kg dose groups but not in the 8 mg/kg group. The weight of the thymus gland in females was not altered at any dose level.

Note: Organ weights appear to be reported for only 10 males and 5 females/group without explanation. Since 20 animal/sex/group were used in the study, an explanation would be in order.

Note: In the discussion (p. 11), brain and serum cholinesterase inhibitions are reported for the 8 mg/kg dose group, but no other cholinesterase data appear in the study.

7. A significant reduction in the number of live fetuses/pregnant dam was observed in the 2 mg/kg dose group, but not in the higher dose groups. The implantation rate was significantly reduced in the high-dose group. (table 6, p. 17).

8. One fetus with gross abnormalities (microagnathia, anophthalmia, tongue-tie) was observed in the low dose (2 mg/kg) group. None were observed in the control or higher dose groups.

9. Ventral ceptum defect was increased in dosed groups and shows a tendency toward a dose response. One case of transposition of cardiac blood vessels was found in each of the 2.0 mg/kg and 4 mg/kg dose groups. Defects of the thymus were identified in a small percentage of fetuses from all dose groups, but not so in the controls.

10. Compound was administered by injection rather than orally.

11. Core Classification: Supplementary

Comments:

There are certain passages in the document which lack clarity. Presumably this is due to difficulties inherent in language translation from Japanese into English. Improved renditions of the following passages would be desirable, but may not be necessary: paragraph at the top of page 4, paragraphs at the bottom of page 7 continuing onto page 8, the next paragraph on page 8 and the last paragraph on page 10.

Another point of concern is that the method and materials section indicate there were 20 rats/sex/dose group at the beginning of the study. When discussing mating performance on page 7, the report indicates that "To the male rats that did not copulate, that is, one from control group, 3 from 2.0 mg/kg group, 2 from 4.0 mg/kg group and 7 from 8 mg/kg group ....." This was interpreted by this reviewer to mean these numbers of male rats out of each group of 20 "failed" to copulate. Yet, in the discussion on page 10, the report indicates "As for reproductive status, a decline in mating index was found among the male rats of the 8.0 mg/kg group. In 7 out of 10 cases of male rats, copulation was not seen." This is not clear; obvious "failure" of 7/10 rats to copulate is more serious than 7/20 "failure."

Adverse effects on a number of parameters were identified which were more pronounced for males than for females. This is probably due to the fact that males were dosed for a much longer period of time. The parameters affected in males of greatest concern were body weight, reproductive function, thymus weight and spleen weight. Changes in all of these parameters are those of concern upon exposure to dioxins and chlorinated phenols, due in the latter case to dioxin contamination. (Faith and Moore, 1977; Casarett and Doull, 1980). Hence, such observations raise concerns about possible contamination of NTN 8629 with dioxin. An analysis for composition of NTN 8629 used in this study was not provided, and must be provided.

NTN 8629 appeared to exert toxic effects at the lowest dose, 2 mg/kg, employed in this study and a no-effect level was not established.

References

Casarett and Doull's Toxicology (1980) 2nd edition MacMillan Publishing Company, Inc., NY. Pages 343, 389-90.

Faith, R.E.; and Moore, J.A. (1977): Impairment of Thymus Dependent Immune Functions by Exposure of the Developing Immune System to 2,3,7,8,-tetrachlorodibenz-p-dioxin (TCDD). J. Tox. Env. Health, 3 p. 451.

Study Title: Administration to Female Rats During Peri- and Postnatal Periods.

Laboratory: Institute for Animal Reproduction, Fukaya 1103, Dejima-Mura, Niiharu-Gun, Ibaraki-Ken.

Date: Tab 20, July 21, 1977

Accession Number: 073098

Material Tested: NTN 8629 developed by Nihon Tokushu Noyaku Seizo Co. Ltd.

Animals: Wistar Imamichi SPF Rat

The stated purpose of this study was to investigate the effects of NTN 8629, administered daily from the 17th day of pregnancy through the 21st day of nursing, on the course of pregnancy during the perinatal period, delivery, nursing, and developmental periods of the F1 generation.

Materials and Methods: (Quoted from Page 3)

"1. Animals tested and maintenance:

Nulliparous Wistar Imamichi SPF rats, 80 days after birth, were kept in a week of conditioning course and then individually transferred into a cage with a male rat for mating in the late afternoon when they showed a sign of proestrus in the vaginal smear. Female rats with which the vaginal plug or sperma was detected were used in the experiment as animals in the pregnancy of 0 day. They were kept in a breeding room air conditioned to 25 ± 1 °C and received the Rat/Mouse Solid Diet for Raising, supplied by Funabashi Farm Co., Ltd., and water ad libitum.

"2. Experimental grouping and doses:

Pregnant rats were divided into 4 groups, each comprising 20 animals, for administration at the following 4 dosis levels as determined on the basis of the results from a preliminary experiment*:

Control group	0 mg/kg daily
Low dose group	0.5 mg/kg daily
Median dose group	1.3 mg/kg daily
High dose group	3.38 mg/kg daily

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* In the same procedure as described for the full experiment, pregnant rats were administered with the compound at 5 dosis levels, 0, 0.8, 4.0, 20 and 60 mg/kg and the dosis groups were compared to the control group to see how the status of pregnancy at the final stage of pregnancy, delivery condition, nursing behavior and pups were affected."

The remainder of this materials and methods section is paraphrased from the corresponding section of the study report.

NTN 8629 was emulsified in 1 percent aqueous Tween-80 and was administered by injection subcutaneously in the dorsal neck area using a constant volume of administration of 0.25 mL/100 g body weight. Controls were similarly injected with the solvent only.

Three days post partum, pups were adjusted to 4 males and 4 females/dam. Pups were weaned 21 days after birth. The examination schedule is described as follows:

1. Examination of Dams

   a. General condition was observed during the course of administration.

   b. Periodic weighings were performed during pregnancy and lactation.

   c. Food and water intake were measured during pregnancy and lactation.

   d. Gross pathological examination was performed on F_0 dams at weaning. The following organs were removed from 10 dams/group and weighed: liver, kidneys, spleen, heart, brain, pituitary gland, adrenal glands, thymus, thyroid glands, ovaries and uterus.

2. Examination of F_1 Generation

   a. At delivery offspring were examined to determine the number of live and stillborn pups, number of malformations, frequency of atelectasis (collapsed lung) and sex ratios.

   b Pups were weighed periodically during lactation. Estimates of the 3-day survival index and weaning index (21-day survival) were made.

   c. Growth and development of 10 litters/group were assessed as follows:

      1. On the 3rd day of lactation, the percent of pups with bilateral auricular stand (language translation problem) was determined.
      2. The number of days for pups to achieve the following were determined: complete protrusion of both the upper and lower incisors from the gum; hair growth, i.e., time to achieve complete coverage of hair in the neck region; and time by which eyelids were completely opened, bilaterally.

d. Reflex and motor function tests were performed on all surviving pups on the day of weaning. These tests included righting reflex, corneal reflex, Preyer's reflex (auricles jerk with sounds, translation problem), and grip test.

e. On the day of weaning, one male and one female from each litter were sacrificed by anesthesia and autopsied for gross pathology. This and additional evaluations conducted on the weaned pups (F1 generation) are depicted in the following diagram as it appears in the study report, page 7:

F_0	F_1	
Each dam	4 female pups	1. Reproductive function test. 1. Autopsy 10 weeks after birth. (for sexual maturity) 1. Reared up to 10 weeks after birth. 1. Autopsy at weaning.
	4 male pups	1. Reproduction function test. 1. Autopsy 10 weeks after birth. (for sexual maturity determination) 1. Bright/dark discrimination learning. 1. Autopsy at weaning.

f. Pups were reared for 10 weeks postweaning, and during this period were examined for the following: general observations, body weights of 3 rats/sex/group determined weekly, food and water intake. Ten weeks after birth, sexual maturity was scored on one male and one female F1 generation rat from each of 10 F0 dams. These assessments involved sacrifice of the animals followed by autopsy for gross pathological examination. Among males the penis was examined and scored according to type, i.e., V type (undifferentiated), W type (immature stage) or U type (mature stage). Maturity among females was assessed according to the age at which vaginal opening occurred and the time at which the estrus cycle recurred two or more times.

g. Gross pathological examinations were performed 10 weeks after birth on a male and female rat (F1 generation) from each litter. This involved sacrifice with anesthetic followed by autopsy for gross pathologic observation. Organs weighed and examined included: liver, kidneys, spleen, heart, brain,

pituitary, gland, adrenal gland, thymus, thyroid glands, and testes or ovaries.

h. Reproduction function tests on F1 pups conducted 10 weeks after birth involved mating a male and female pup from each litter. Among the dams of each group, 10 were sacrificed on the 14th day of pregnancy for a counting of corpora lutea and implants. An estimate was made of the implantation index (implants/corpora lutea) x 100. Further, viable and dead embryos were counted.

   Five dams from each group were allowed to deliver and nurse their pups for periods up to 3 days post-delivery. Also, maternal animals were sacrificed and autopsied to determine the number of copora lutea, implants and to weigh "major visceral organs."

i. The methods and materials section also describes the performance of learning tests. This was not a part of the previous studies as presented. Animals used in this study apparently were rats of the male sex only. Dose groups were 0, 0.5, 1.3, and 3.38 mg/kg. The manner and frequency of administration of the test compound is not clear. The purpose of this study was to determine the possible influence of NTN 8629 or memory as measured using a Skinner Box. Details of the learning and testing procedures are found on page 9 through 11 of the study report.

Results:

1. The first table (p. 23) to be found in the results section consolidates information evidently on the preliminary experiment as mentioned in the methods and materials section. The information as presented in the table covering 5 pregnant rats/dose group show that days of pregnancy, parturition index, litter size, survival index (pup) were not affected by doses of 0.8 and 4.0 mg/kg. Inhibition of cholinesterase occurred at all doses and was dose dependent:

	% Cholinesterase Inhibition	
Dose, mg/kg	Serum	Brain
0.0	0.0	0.0
0.8	5.0	13.6
4.0	33.7	48.5
20.0	68.3	80.6

The number of maternal deaths increased with increasing dose, i.e., 0/5, 1/5, 2/5, 4/5, and 5/5 at the respective

doses of 0, 0.8, 4.0, 20.0, and 60.0 mg/kg. (Mean litter size was adversely affected at 20 and 60 mg/kg). Pup survival index was markedly reduced at 20.0 mg/kg.

2. In the principal study, the mean body weights of the 20 rats/group as determined for the 20 days of pregnancy and 21 days postpartum (nursing) were not affected in the dosed groups (table 1. p. 24).

3. Food and water intake during the pre- and post-natal periods did not appear altered by any dose level.

4. Absolute organ weight determinations revealed the following: a) an apparently dose-related increase in spleen weight which reached statistical significance in the high dose group. Heart weight of low and high (but not middle) dose groups was greater than in the control. Weight of the thymus was not affected by dosing. There were no other remarkable effects on organ weights of F0 dams (table 4, p. 27).

   With regard to the effects noted on spleen weight, the same observation was made on male rats reported in the study entitled, "Administration Before and During the Early Stage of Pregnancy." Therefore, it is likely that NTN 8629 induces an increase in spleen weight. The no effect level appears to be the 0.5 mg/kg dose (table 4, p. 27).

   No gross or histopathology data are presented.

5. Reproduction Performance:

   None of the factors monitored in relation to reproduction performance was altered in dosed groups. These included duration of pregnancy, number of pups delivered (surviving or dead), pup sex ratio, male and female pup survivor weights and pup 3-day and 21-day survival periods (table 5, p. 28). There were no abnormal findings with respect to pup external appearance reported.

6. F1 Generation pup weights during 21 days of lactation were not altered in the dosed groups (table 6 page 29).

7. Growth and differentation of lactated pups, as appraised by inspection for auricular stand, eruption of incisors, hair growth and opening of eyes, were not altered in dosed groups (table 7, p. 30). The authors also note that no adverse findings were observed with regard to righting, Preyer, and corneal reflexes and in gait condition. There was no evidence of abnormal behavior among F1 pups.

8. Body weight gain and sexual maturity data did not reveal any remarkable compound related effects, possibly excepting a somewhat delayed, but significant, glans development (tables 7 to 8, pp 30 to 31).

9. Food and water intake and efficiency of food utilization were not affected in the F1 generation rats of either sex. This was essentialy true also in F1 females during 20 days of pregnancy, possibly excepting food consumption and water intake in the high dose group where both parameters were numerically reduced, though not reported as statistically significantly reduced (tables 10 through 12, pp 33 to 35; 16 to 18, pp. 39 to 41).

10. Gross pathologic examination of F1 generation rats 10 weeks after birth revealed no abnormal findings. It should be noted that no autopsy or histopathology data has been submitted in support of this claim, and consequently the claim cannot be accepted. There were no remarkable dose related changes in organ weights with the possible exception of the thymus. In both sexes, particularly females, the weight of the thymus gland in dosed groups numerically exceeded that of the control. However, such increases were not statistically significant (tables 13, 14 pp. 37, 38).

11. Parameters related to reproduction in the F1 generation revealed successful reproduction with no significant dose related adverse effects on any of the parameters examined (we should note that the index of live embryos was significantly increased in all dosed groups, but this may be attributable to an unusually low figure for the control, table 19, p. 42).

12. Data obtained with respect to learning patterns indicate that exposure to NTN 8629 did not compromise rat learning of the light and dark discrimination tasks (table 21, p. 44).

Conclusions:

1. Results of the preliminary experiment conducted at doses of 0.0, 0.8, 4.0, 20.0, and 60.0 mg/kg demonstrate cholinesterase (brain and serum) inhibition at the lowest dose (0.8 mg/kg).

2. The preliminary study illustrated a dose-related adverse effect on maternal survivability, which appeared to extend to the lowest dose employed, 0.8 mg/kg. Litter size and pup survival index were adversely affected at 20.0 mg/kg.

3. In the primary study, spleen weight of F0 dams at sacrifice was observed to increase in a dose-related manner which reached statistical significance in the high dose group. This effect was not evident in the F1 generation. No effect level for the generation F0 was 0.5 mg/kg.

4. The weight of the thymus gland was not altered by NTN 8629 administration in the F0 generation. In the F1 generation, the weight of the thymus was numerically increased in dose groups, but not with statistical significance.

5. With the exception of a small but significant delay in glans development observed in the high dose group of the F1 generation, there were no adverse effects identified on the various reproductive parameters evaluated in either the F0 or F1 generations.

6. No adverse effects of NTN 8629 were identified in the limited neurological and learning tests performed on the F1 generation.

7. No adverse effects on weight gain, food consumption or water consumption was observed in either F0 or F1 generations.

8. Claims of no gross pathologic findings were not substantiated by data submission of either gross or histopathologic data. It should be noted that the very last page (87) of the study contains photographs of an interventricular septal defect (0 mg/kg) and dwarfism (20 mg/kg). These are presented without comment. This reviewer is persuaded that this indicates dwarfism in the 20 mg/kg dose group of the preliminary study. Petitioner should be advised to submit data and comment on this dwarfism.

9. The composition (purity) of the NTN 8629 sample employed in this study was not specified. Petitioner should be advised to submit an analysis of the material's composition.

10. Route of administration of NTN 8629 was by subcutaneous injection. Oral administration is required.

11. Invalid. Upgrading is dependent upon submission of adequate information to address the data deficiencies indicated above including histopathologic data.

Study: NTN 8629 - Multigeneration Study in Rats

Laboratory: Institute of Comparative and Human Toxicology, Albany Medical College, Albany, NY and International Center of Environmental Safety, Albany Medical College, Holloman AFB, New Mexico.

Study Number and Date: Tab 3, February 1, 1978

Accession Number: 073093

Material Tested: NTN 8629 Technical (93.2%)

Animals: CD (Long-Evans) Rats

The method and materials for this study are quoted as follows from the study report:

"A. Test Compound

NTN 8629 technical by the Bayer A.G. was a premix with the inert material Wessalon S (synthetic silica) 1:1.

"B. Experimental Animals and Their Maintenance

Two hundred pathogen-free, CD (Long-Evans) rats were acclimated to laboratory conditions and were divided into four groups in which the animals had approximately equal body weights. The age of the animals at the beginning of the study was 44 days. The dosage groups were:

Group	Dietary Level (ppm)	No. of Animals Male	Female
I	0	25	25
II	5	25	25
III	50	25	25
IV	500	25	25

"The animals were maintained in air-conditioned quarters with food and water available ad libitum. Except during the mating period, they were housed three per cage, male and female cages alternating. The diet was Wayne Lab-Blox Mash.

"Dietary mixtures were prepared once a week in a Hobart laboratory mixer. Each fresh mixture was assayed to assure that the proper dietary levels of the NTN 8629 were maintained."

C. Chronological Sequence of This Study

Note: Information presented in this section of the Methods and Materials is incompatible in many aspects with the data presented in the body of the study. Accordingly, the following descriptive passages by the study directors as to the course of the study are interrupted periodically by this reviewer's comments, for purposes of clarification.

"Animals of the F0 generation were maintained on their respective diets for 84 days prior to the first mating. The offspring of this mating (F1A) were reared to 30 days postpartum, sacrificed, and subjected to gross examination for teratological effects."

Comment: Results on the gross examination of the F1A rats were not submitted.

"Approximately 10 days after the weaning of the F1A pups, the F0 parents were remated for a period of 19 days. On day 20, considering the first appearance of sperm in the vaginal smear as day 0, five females of each dosage group were sacrificed. The unborn pups were examined for teratological effects. The remaining dams were allowed to rear their young to approximately 21 days postpartum when 25 males and 25 females, generally healthy pups closest to mean litter weight, were selected to establish the F1B parent animals. F0 parents and surplus F1B pups were sacrificed and examined macroscopically." [p. 5.]

Comment: Data for surplus F1B pups were not submitted.

"The selected F1B generation rats were maintained on the NTN 8629 containing diets for approximately 85 days before mating to produce an F2A generation.

A repetition of this regimen produced the F2B generation with the above observations and tests repeated. In addition to the gross and microscopic teratological examinations, cholinesterase assays were performed on blood and brain tissue from F2A pups." [p. 5.]

Comment: There is cholinesterase (erythrocyte, plasma, and brain) data on the F2A generation, but no gross or microscopic examination data for the F2A animals as claimed.

"When the F2B generation was approximately 21 days of age, 25 males and 25 females were selected from each dosage group for mating."

Comment: Although there were more than 25 males and 25 females in Group I (Control) of the F2B generation, there were only 14 males and 15 females in Group II, and 25 males and 21 females in Group III and no offspring in Group IV.

The F2B animals, "were maintained on their respective diets for approximately 3 months and then were mated one male to one female for 19 days. The resulting F3A generation was reared to approximately 28 days postpartum, sacrificed, and subjected to detailed histopathological examination. Blood was taken for cholinesterase assays." [p. 5.]

Comment: There is cholinesterase (erythrocyte, plasma, and brain) data on the F3A generation. In addition there are gross observations on F3A generation rats, but microscopic data exist only for five rats/sex/dose, in groups I to III. (Vol. 6, Tab 3,V.)

"Approximately 20 days after weaning the F3A pups, the F2B generation was remated. Five pregnant dams in each group were sacrificed at day 20 for teratological examination. Pups of the F3B generation were reduced to 25 males and 25 females in each dosage group and were maintained for approximately 3 months. They were then sacrificed and examined macroscopically and microscopically. Brain and blood cholinesterase determinations were made." [p. 6.].

Comment: Five pregnant F2B dams/dose were sacrificed and examined as described. The total number of F3B generation offspring in each group was with one exception less than 25. These numbers in each group were: Group I (24 males, 28 females), Group II (1 male, 4 females), and Group III (2 males, 5 females). There were no Group IV animals.

There is cholinesterase data and gross pathology on the limited number of F3B animals. However, the number of animals examined microscopically from each group was: Group I (3 males, 3 females), Group II (1 male, 4 females), and Group III (1 male, 5 females).

"Approximately 10 days after weaning the F3B pups, F2B parents were again mated to produce the F3C generation. At 21 days postpartum F2B parents were sacrificed for detailed microscopic examination with brain tissue and blood taken for cholinesterase assay. F3C pups in excess of 25 males and 25 females for each group were also sacrificed." [p. 6.]

Comment: Only gross observations of the F2B generation were provided, but no microscopic data. There is cholinesterase data for the F2B generation as described.

"F3C pups in excess of 25 males and 25 females for each group were also sacrificed." [p. 6.]

Comment: There were no F3C pups in excess of 25 in any group.

"The F3C generation rats were fed their respective diets for approximately 1 month. They were then sacrificed for detailed histopathological examination with cholinesterase activities also determined." [p. 6.]

Comment: The number of F3C generation rats in each group initially was: Group I (13 male, 23 female), Group II (none), Group III (10 male, 5 female), Group IV (none). There is very limited cholinesterase data on the FC3 animals. There is gross macroscopic examination data on some of the animals. Microscopic examination data is limited to the following numbers of animals in each group Group I (0 male, 3 female), Group II (none), Group III (0 male, 1 female).

If Section D of the Methods and Materials was intended to convey to the reader the proposed course of action to be pursued in the study, it should not have been composed in the past tense. Furthermore, it should have included qualifying statements describing what actually was accomplished.

Results:

A. F0 Generation

Weekly weighings of F0 generation animals revealed that growth rates of rats of both sexes were depressed in Group IV, but not in the other groups. Food consumption during the premating period was increased only in Group IV females. Toxic signs including piloerection, hyperactivity, tremors, and hypersensitivity to sound appeared after 6 to 8 weeks and continued through two matings and until sacrifice in Group IV animals, particularly in females. Presumably such signs were not observed in the other dose groups. NTN 8629 in the diet did not appear to have any effect on mortality of the F0 generation.

B. First and Second Mating of F0 Generation
(♂ F1A and ♂ F1B)

The study report indicates that there was no effect of NTN 8629 on F0 fertility during the first and second matings at any of the dose levels when compared to control values. This appears to be true (table 5). It should be noted, however, that there was a big decline in fertility in all groups, controls included, in the second F0 mating.

Dams in Group IV pregnant with F1A and F1B generations experienced reduced growth rates during pregnancy. At certain time points after pregnancy there were significantly lower body weights in Group III also, though not at the extreme time point of 21 days. Group II animal weights were not affected (table 6).

"NTN 8629 in concentrations up to 500 ppm had no effect on the gestation period of F0 females after both matings." [table 7].

The sizes and weights of F1A and F1B litters born to dams in Group IV were less than those of comparable controls (tables 8-11). Furthermore, there were marked declines in the number of Group IV offspring surviving during the course of the 21-day postpartum period (tables 8 and 10). Mean litter weights of Group IV animals also showed marked declines with respect to controls (tables 9 and 11). Group III litter sizes were normal in both F1A and F1B generations. Group III F1B mean litter weights exhibited a continuous time dependent upward trend with respect to controls during the 21-day postpartum period (table 11). Though this table does not indicate changes at any of the time points to be significant, our independent calculations show that at 21 days postpartum, the increased mean litter weight for Group III was significant at $p < 0.05$.

F1A and F1B pup weights were depressed in Group IV, but not in the other groups during the 21-day postpartum (Table 12 and 13). The number of stillborn pups in any dose group for either the F1A or F1B generations was unaffected by treatment (Table 14).

Pup mortality for F1A and F1B generations in Group IV at 21 days postpartum (F1A, 79%; F1B, 66%) was in excess of that of controls (F1A, 4%; F1B, 12%). The study authors advise that pup deaths were accompanied by the cannibalization of the pups by the mothers, suggesting innate pup defects recognized by the mothers (see p. 23). Pup mortality was unaffected in other dose group.

C. F1B Generation

The body weights of F1B generation male and female rats of Group IV were less than the respective control weights during the 14-week premating period. No effect was observed in the other groups (tables 17 and 18). Food consumption for the F1B rats, male and female, in Group IV was significantly lower than that of the control at a number of time points during the 12-week observation period, indicating a compound-related effect. There were no compound-related effects on food consumption in the other dose groups (tables 19 and 20). Mortality rate in the Group IV F1B generation rats appeared to be high among the few offspring in this group that survived weaning. Thus, the high mortality seen from birth throughout the course of study affirms an adverse effect of the compound on the F1B generation.

Effects on Spermatogenesis in F1B Rats

Of ten rats total from the F1B Groups I to IV in which the testes were examined microscopically, the single Group I rat and three Group II rats examined were within normal limits, but the four Group III rats were described as having slight spermatogonial karyopyknosis and slight chromatin dispersion (margination) in the primary spermatocytes. The two Group IV rats had moderate spermatogonial karyopyknosis with slight chromatin dispersion in primary spermatocytes. Thus 100 percent of animals in Groups III and IV which were examined, exhibited these phenomena, and there were too few animals examined in the other groups to confirm its presence or absence. (See microscopic changes for F1B generations, p. 104.)

D. First and Second Matings of the F1B Generation

The F1B mating generations in Group IV consisted of only three males and six females. Every reasonable effort was made to mate these animals, and while mating was confirmed by vaginal smear, such matings did not yield offspring in either the F2A or F2B generations. There is the suggestion of reduced fertility in the second F1B mating of Group II, both with respect to the control (40 vs 52) and with respect to the first breeding in Group II (40 vs 60) in terms of fertility rate (Table 5). Neither the gestation period nor body weight of F1B females during the F2A and F2B pregnancies were affected by 5 and 50 ppm NTN 8629 in the diet (Tables 7 and 21). According to study authors:

> "F2A litters born to mothers which had received 50 ppm NTN 8629 were smaller and weighed less

than control litters only on the day of birth, but were normal in both respects by day number 4." (P. 12, see tables 23 and 44.)

However, F2A generation Group II animals exhibited a significant ($P < 0.05$ by our calculations) reduction in mean litter weight by the 21st day postpartum. This trend was also observed for Group II at 4 and 12 days (table 23). Further, data on mean litter size suggests a decrease for the Group II F2A generation (table 22).

F2A and F2B generation pup weights did not differ from those of control animals during the 21-day postpartum period (tables 26 and 27). Hence, the reduction in mean litter weight of the F2A generation, Group II, would be attributable to the reduced litter size of this group.

NTN 8629 had no effect on the production of stillbirths in the F2A and F2B generations (tables 14 and 15).

The mean body weights of F2B generation male rats in the first 22 weeks was unaffected by 50 ppm NTN 8629 in the diet. However, male rats in Group II began showing increased body weights by week 7. For weeks 24 through 38, Groups II and III show increased body weights. This phenomenon was not observed in F2B females (tables 32 and 33).

The mortality of the F2B generation at the time of sacrifice was unaffected by 5 or 50 ppm prothiophos in the diet. It should be noted, however, as mentioned previously that there were only 14 male and 15 female rats in Group II after weaning, considerably less than the 25 sought. There also appears to be a discrepancy in the petition data as to the number of F2B Group II animals after weaning: Volume 6, Tab III, Page 10 shows the total number of male and female rats at weaning in the 5 ppm group to be 8 and 20, respectively. This contrasts to the 14 males and 15 females indicated for the group in Volume 2, table 32 and on page 104 item C. The error probably exists in Volume 6 but must be clarified.

E. F2B Generation Matings

Fertility for the F2B females through three matings showed substantial decline subsequent to the first mating (table 5).

"Table 5 (Reproduced from page 34)

NTN 8629

Fertility Rates[a] in F0, F1B, and F2B Generation Rats Fed NTN 8629 in the Diet (in %)

Generation		Concentration in Diet (ppm)			
		0	5	50	500
F0 x F0	F1A	76	80	75	68
	F1B	47	59	50	44
F1B x F1B	F2A	64	60	50	0
	F2B	52	40	53	0
F2B x F2B	F3A	96	87	81	
	F3B	39	53	24	
	F3C	41	0	25	

[a] Fertility rate = $\frac{\text{Number of litters born}}{\text{Number of females mated}} \times 100$"

Fertility was apparently unaffected for Groups I to III in the first F2B mating. It should be noted that decline in fertility in all groups was marked from the first to the second F2B breeding in the control and dose groups. As a general observation, the second breeding of given parent animals is normally characterized by fertility rates which are higher or certainly equal to those of the first breeding. The contrary findings in the present study are suggestive of poor husbandry and quite possibly could have effectively masked responses otherwise observable as a consequence of dosing. On the other hand the decline in fertility of control animals suggests all animals were under common stress, which might be expected to enhance adverse responses to prothiophos. In this regard, the third F2B mating of Group II animals was unproductive, suggesting an adverse reproduction effect of prothiophos at 5 ppm. This and other effects in the 5 ppm group indicate this dose level had an adverse effect in reproduction.

Fertility in the second and third matings of Group III animals was markedly down.

F. Comments on the F3 Generation

It is evident from data presented in table 41 that for the F3A generation, neither 5 nor 50 ppm prothiophos had any effect on litter size for periods up to 21 days postpartum, at which time the animals were sacrificed. The same cannot be said of the F3B and F3C generations. With respect to the F3B generation, the study report indicates that the 5 ppm group exhibited significantly reduced litter sizes at days 4, 12, and 21 postpartum as shown in table 42, (next page). Though perhaps not statistically significant at birth, as claimed by the authors, the mean litter size at birth for the 5 ppm group was considerably less, numerically, than that of the control, 3.9 vs. 7.8. It is noted that Table 42 does not provide the necessary statistical parameters for independent confirmation of the significance of differences between groups.

Litter weights at various time points postpartum for the F3B, 5 ppm dose group, were significantly less than those of controls (table 46).

"Table 42 (Reproduced From p. 75)

Mean Litter Size of F3B Generation From F2B Generation Dams Exposed to NTN 8629 in Utero, For Twenty-one Days Postpartum and Then Fed NTN 8629 in the Diet for Six Months.

Concentration Diet (ppm)	NUMBER OF DAYS POSTPARTUM							
	0		4		12		21	
	A[a]	AD[a]	A	B[b]	A	B	A	B
0	7.8	.11	6.6	7.4	6.0	7.7	5.9	7.6
5	3.9	1.00	2.8	3.7	.9	1.8	.6	2.5
50	6.4	.20	5.2	6.5	1.4	3.5	1.4	3.5

[a] Mean A = $\frac{\text{Number of young surviving at 0, 4, 12, and 21 days}}{\text{Number of litters born}}$

Mean A_D = $\frac{\text{Number of stillbirths}}{\text{Number of litters born}}$

[b] Mean B = $\frac{\text{Number of young surviving at 4, 12, and 21 days}}{\text{Number of litters surviving at 4, 12, and 21 days}}$ "

The 50 ppm dose group of the F2B generation showed marked declines in litter size at 12 and 21 days postpartum but not at earlier time points. A time dependent decrease in litter weights of the F3B generation of Group III, being significant at 12 and 21 days, serves to reinforce a conclusion that 50 ppm prothiophos exerts an adverse effect on reproduction (table 46).

In the F3C generation, Group II (5 ppm) animals failed to reproduce, though mating was affirmed, demonstrating an adverse effect of prothiophos. As in the case of the F3B generation there was a marked loss of Group III pups by days 12 and 21 postpartum in which statistical significance as before could not be determined (table 43). Litter weights of Group III rats did not appear to be affected (table 47).

G. Teratological Data

No significant differences were noted between control and dose Groups II and III among F0 dams with respect to: (a) number of corpora lutea, (b) number of resorption sites (although Group III was 2.2 ± 3.4 vs 1.0 ± 1.2 for control), (c) number of viable young, d) litter weight, and (e) mean pup weight. However, Group IV animals showed a significant decrease in corpora lutea (10 ± 0.8 vs 12.2 ± 1.6) (table 62).

Similarly, no significant differences were noted in parameters a-d named above between controls and groups II or III for F1B dams. There was a substantial numerical difference in mean litter weight between control (7.2 ± 11.3) and Group III (1.4 ± 1.9) (table 63).

F2B dams pregnant with the F3B generation did not exhibit significant differences between control and dosed groups II and III. In fact, all comparisons from group to group are remarkably close (table 64).

Volume 6 - Necropsy

Reviewed all necropsy sheets on F3A, F3B, and F3C generations. There were no remarkable findings.

Review of Histopathological Examination Beginning on Page 100 of Volume 2

Histopath examination summarized as follows:

A. Gross Observations of the F0 Generation

Approximately 25 rats/sex/dose were examined grossly; no remarkable differences from control.

B. Gross and Microscopic Observations of F1B Generation

Approximately 25 rats/sex/Group (I, II, III), plus 4 male and 10 female rats/Group IV were examined only grossly (p. 103). Findings were not remarkable.

Ten male rats total from Groups I (1 rat), II (3 rats), III (4 rats), and Group IV (2 rats) were examined microscopically (testes only). Results demonstrated an increase in Groups III and IV for spermatogonial karyopyknosis and chromatin dispersion. Microscopic examination of the ovaries of four F1B females (one from each group) were within normal limits. (p. 104.)

C. Gross observations of the F2B, F3A, F3B, and F3C generations were unremarkable with the exception of a large number of animals reported to have pneumonia, particularly in groups F2B, F3B, and F3C, in control and treated animals.

Summary of Cholinesterase Effects

Group IV (500 ppm) rats exhibited toxic signs including hyperactivity, tremors, hypersensitivity to sound, and piloerection. Cholinesterase activity was not measured in Group IV rats. An association between these toxic signs and cholinesterase inhibition might be reasonable, though such signs could also be viewed as evidence of neurotoxicity of varied etiology. The latter possibility renders a neurotoxicity study more imperative.

Cholinesterase determinations were not reported for the F0 or F1 generations.

A. Cholinesterase (plasma, red cell, and brain) determinations, were made on F2A generation pups at weaning. These data show that plasma and red cell cholinesterase were not affected by 5 or 50 ppm prothiophos in the F1B dam diet. Brain cholinesterase values for Group III (50 ppm) male and female pups were elevated when expressed on a μ/g brain protein basis, but not when expressed on a μ/l brain homogenate basis. Further analysis showed that the mg protein/ml brain homogenate for Group III animals was significantly ($p < .05$) reduced with respect to controls. This served to explain the elevated brain cholinesterase as expressed on a per brain protein basis. Thus in consideration of such findings, it appears that none of the cholinesterases assayed on the weanling F2A generation pups was measurably affected by prothiophos at 5 or 50 ppm

(tables 28-31). However, the fact that brain levels are reduced with dose is important and requires explanation.

B. All three cholinesterases were assayed in F2B generation rats. These animals had been exposed in utero for 3 weeks postpartum and for 39 weeks in the diet. Results show that significant inhibition occurred only in the case of red cell cholinesterase for male rats in Group III (50 ppm). The magnitude of inhibition was not great: 1.65 vs 2.17 μ/ml (24% inhibition). Though not statistically significant there was a lowering among females of the same group: 1.86 vs 2.27 μ/ml (18% inhibition) (table 36-39).

C. Plasma and red cell cholinesterase activity were measured in the F3A generation at weaning and found to be comparable to controls for animals of both sexes in Groups II and III (table 51).

Plasma, red cell and brain cholinesterases were assayed in F3B and F3C generations after exposure in utero, 3 weeks postpartum, and then to prothiophos via the diet for approximately 1 month. Plasma and red cell (68 and 22 percent, respectively) cholinesterases were significantly inhibited in Group III females but not in Group II females. Brain cholinesterase was not inhibited in Group II or III females. Unfortunately, there were not enough male rats in the F3B generation to obtain meaningful information on cholinesterase effects. Also, in the F3C generation there were not enough animals in Groups II and III for the evaluation of cholinesterase effects (Tables 56 and 57).

Conclusions to be drawn from the cholinesterase data are: 1) weanling rats from dams exposed to prothiophos during pregnancy and during the weaning period did not exhibit cholinesterase inhibition, suggesting that the cholinesterase inhibitor was not significantly transferred via dams milk, 2) rats first exposed in utero, then during the 3-week postpartum period and for additional periods of time of a month's duration or longer exhibit inhibited plasma and red cell cholinesterase at 50 ppm (percentage inhibition as discussed above), but not measurably, so by 5 ppm prothiophos. Brain chlolinesterase is not inhibited by 5 or 50 ppm prothiophos under these circumstances.

Conclusions

1. Toxic signs including hyperactivity, tremors, hypersensitivity to sound and piloerection were observed in animals receiving 500 ppm prothiophos in the diet. No such signs were observed in lower dose groups. A neurotoxicology study is indicated.

2. Plasma and serum cholinesterases were inhibited by 50 ppm, but not by 5 ppm prothiophos in the diet. Brain cholinesterase was not inhibited by 50 ppm prothiophos in the diet. Rats weaned from dams fed 50 ppm prothiophos in the diet exhibited no inhibition of plasma, erythrocyte, or brain cholinesterase.

3. FO and F1B generation rats in the high-dose group experienced decreased growth rates which continued through the 14 weeks of observation. By virtue of the study design F1B rats were exposed pre and postnatally. The study authors offer no explanation for this apparent effect of prothiophos on growth rate, but note that food consumption was essentially normal and that reduced weight gain was not due to reduced food intake. The effect was probably due to altered absorption, metabolism, or utilization of food stuffs (p. 21).

   As a point of concern we wish to note that Faith and Moore (1977) were able to demonstrate reduced growth rates or "runting" among rats in response to dioxin administered prenatally and/or postnatally, where doses of 5 µg/kg of TCDD were administered to dams or offspring at prescribed intervals. The growth rate inhibition mimicked that induced by neonatal thymectomy, neonatal treatment with corticosteroids or injection of neonatal animals with allogenic lymphocytes. All these produce thymic dysfunction. This finding is being cited due to possible prothiophos contamination by dioxin. Histopath sheets, in the prothiophos study, however, do not identify any effects on the thymus.

4. Although fertility rates (Table 5) of 500 ppm dosed rats for the first generation was unaffected, litter sizes (Table 44) were markedly reduced. Those offspring (F1B) failed to reproduce. Other parameters adversely affected in the 500 ppm group were: Body weight, growth rate, litter weight, and pup weight.

5. At the 50 ppm level, prothiophos appeared to be associated with reduced litter size in the F2A generation and reduced survivably in the F3B and F3C generations.

6. In the F2A generation, Group II (5 ppm) but not Group III (50 ppm) animals exhibited a significant ($p < 0.05$, by our calculations) reduction in mean litter weight by day-21 postpartum, a trend observed from day 4 (table 23). This is indicative of a low-dose effect of prothiophos.

7. In the F2B generation, male animals in Groups II and III exhibited increasing body weights with respect to controls during a 38-week observation period. There was a much earlier onset in Group II (week 7) than in Group III (week 24), suggesting a sex-related effect of prothiophos more profound at the lower dose (tables 32 and 33).

8. Mean litter size for Group II (5 ppm) of the F3B generation was numerically less than that of the control and 50 ppm groups (table 42). As indicated in table 44 this decline in mean litter size of Group II animals was significant at the 4-, 12-, and 21-day postpartum time points.

9. Additional evidence of a more marked adverse effect of prothiophos at 5 ppm than at 50 ppm was the higher mortality among Group II F3B generation pups and the failure of F2B animals of the same group to produce offspring in the F3C generation. Reproduction appears to have been adversely affected at 5 ppm in the F3 generation.

10. Spermatogonial karyopyknosis was observed in 100 percent of F1B Group III and Group IV male rats examined. While not observed histologically in Group II animals, it is possible that less morphologically pronounced, but qualitatively equivalent biochemical responses were occurring in the spermatogonium of these animals. Also, too few Group II animals were examined for this effect. Lack of investigation for this effect among the other generations of the overall study constitutes a serious deficiency. All that can be said at this time is that the response has not been adequately characterized, but is indicative of a serious adverse effect posed by prothiophos even at the low dose of 5 ppm in the diet (page 104).

    It is important to advise that pyknosis has been reported in the literature to occur with increased frequency in rats exposed to TCDD. While not reported in the spermatogonium, pyknosis of the nuclei of cells of the spleen, bone marrow, and renal tubule have been documented by Gupta, et al. (1973).

11. There were too few ovaries examined microscopically for any conclusions to be made.

12. Marked decline in fertility were generally seen in all groups, controls included, upon repeated breeding of the same parents of the FO, F1B, and F2B generations (table 5). As a general finding, the second breeding of given parents is characterized by enhanced fertility, and fertility certainly should not be reduced by more than 10 percent. Such declines in fertility as observed in this study are all indicative of poor husbandry, and could actually mask the detection of adverse effects in prothiophos-dosed animals. This raises a serious concern for the acceptability of the study.

13. Gross pathologic examination revealed pneumonia in a large fraction of the F2B, F3B, and F3C animals. Since pneumonia was prevalent among the control as well as treated groups, the condition is probably not related to prothiophos in the diet. The prevalence of pneumonia may be indicative of poor husbandry (pp 104-110).

14. There were no remarkable teratological findings other than an apparent absence of ossification in the sternum of some of the Group III fetuses in the F3B generation. The reason for this is not clear, but the finding is nevertheless suggestive of a teratological effect of the compound.

15. Analysis of NTN 8629 for possible contaminants is required.

Comments with respect to conformity to guidelines

The study generally conforms to guidelines with the following noteworthy qualifications:

1. Guidelines indicate toxicity should not be observed at the lowest dose studied. The lowest dose, 5 ppm, does appear to exert an adverse effect on reproduction.

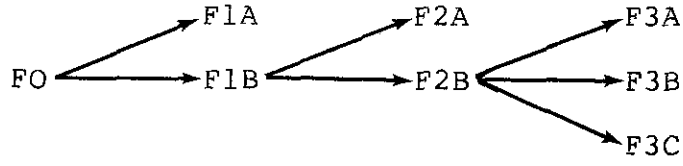
2. Signs of toxicity should be noted for each animal during the course of the study. We are unable to locate any table presenting such observations. Food consumption and body weight data are recorded periodically for each animal (Vol. 6, Appendix 1).

3. Guideline lists a number of reproductive organs which should have full histopathology for control and high-dose group of FO and F1B generations. Actually only certain numbers of animals from the various generations are so examined and only data of a summary nature are presented as opposed to animal-by-animal observations. For example, in F3A, F3B, and F3C

generations no more than five animals/sex are so examined (Vol. 6). For the other earlier generations, only positive findings are noted on an individual animal basis.

4. Organs demonstrating pathology in the FO and F1B generations should then be examined in animals of the other dose groups. Spermatogonial karyopyknosis was observed in the F1B generation in the few animals examined in Groups III and IV. This should have triggered an examination for this effect in all of the F1B males and in those of subsquent generations as well.

5. The study exceeds guideline requirements in that it was carried through three generations where only two are required, and included two matings in each of the first two generations, and three matings in the third, where only one mating is required by guidelines.



Core Classification: Supplementary

Study: A Multigeneration Study of NTN 8629 in Rats

Laboratory: Institute of Comparative and Human Toxicology, Albany Medical College, Albany, NY and International Center of Environmental Safety, Albany Medical College, Holloman AFB, New Mexico.

Study Number and Date: Tab 17, December, 1979

Accession Number: 073093

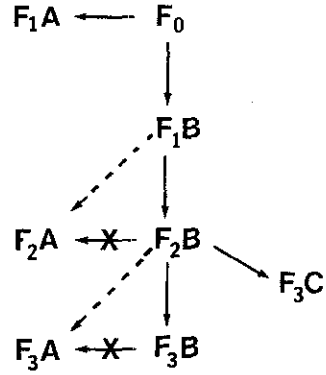
Material Tested: NTN 8629 (purity, source-not indicated)

Animals: CD (Long-Evans) Rat

The methods and materials for this study are presented as follows (including our comments) from the study report.

> "NTN 8629 was administered as an admixture in a diet of ground Wayne Labblox, at levels of 3, 30, and 180 ppm. Control groups were fed ground Wayne Labblox only" (p. 2).

> "This was a multigeneration study which was begun with 200 general pathogen-free CD (Long-Evans) rats which served as the F0 generation. These were divided into groups (presumably 25 rats/group), which were fed control diet or one of 3 diets containing various concentrations of NTN 8629 .... Subsequent generations, derived from this F0 generation were as follows:" (p. 2)



(Note: The diagram as presented in the petition is not consistent with the text (pp. 10 to 11). Corrections we consider appropriate are indicated by lines crossed out and by replacement with broken lines.)

"The rats were housed in an air-conditioned, temperature controlled room which was maintained on a 12-hour light, dark cycle. From weaning to mating, parent animals were housed 5 to a cage with male and female cages alternating. During this mating period the rats were housed on the basis of 1 male to 1 female; subsequently the males were housed 2 per cage and the females

transferred to individual cages for the birth and rearing of litters. They were provided with food and diet cups which were changed three times a week and water from bottles which was changed twice a week. During daily observations, empty feed cups or water bottles were replenished with food and water as required. The rat cages were changed twice a week." (p. 2).

Chronological Sequence of the Study

"Animals of the F_0 generation were maintained on their respective diets for 90 days prior to mating. The animals were then mated on a 1 male to 1 female basis for a period of 19 days. The resulting litters (F_1A) were reared to 21 days post partum, examined and then killed and discarded after first being anesthesized with ether" (p. 3). [Notes: 1) Since the study author does not so state, we will require data showing that generations subsequent to F_0 were always fed the experimental diets; 2) the protocol does not clearly indicate the extent of any examinations carried out on the F_1A generation.]

"Shortly following (approximately 10 days) the weaning of the F_1A litters, the F_0 generation was remated for a period of 19 days.

"Five females in each group were killed, (after first being anesthesized with ether) on day 20 of gestation for teratological examination considering the appearance of sperm in the vaginal smear to be day 0 of pregnancy" (p. 3). (Note: This would leave 20 pregnant dams/group for continuation of the study.)

"The remaining dams were allowed to rear their young to 21 days post partum when 25 males and 25 females were selected from each group to form the basis of the F_1B generation. The (F_0) and surplus F_1B pups were killed after first being anesthesized with ether and then examined macroscopically. Six male and six female rats of the control group and of each dietary group containing NTN 8629 were used to determine blood and brain cholinesterase" (p. 3). [Note: The latter statement does not specify from which generation the 6 animals were taken for cholinesterase determination.]

"The F_1B and succeeding F_2B and F_3A generations were used in a similar manner" (p. 3). [Note: this latter statement is not clear.] "As the F_3B generation did not yield sufficient offspring to produce the required 25 males and 25 females per group, an additional breeding took place to provide an F_3C generation" (p. 3). [Comment: We believe this latter sentence would be clearer rephrased as follows: Since the F_3B generation consisted of less than the required 25 male and 25 females per group, an additional breeding of the F_2B generation took place to provide an F_3C generation.] "After the F_3B and F_3C generations were reduced to 25 males and 25 females per group, they were fed their respective diets for 90 days, then killed, after being

anesthetized with ether, and then subjected to a detailed histopathologic examination and the determination of blood and brain cholinsterases" (p. 3). [Questions: since the number of rats in the F_3B generation was less than 25/group as cited above, should the statement be interpreted to mean that the F_3B and F_3C generations were combined? Such a combining is not appropriate? This requires clarification.]

"All rats were observed daily for evidence of behavioral or physical change. The mean weekly food intake was recorded as follows: Parent rats for approximately 90 days of the premating based on the total food consumed/ number of rats per box. Water consumption was monitored visually and if it appeared to be affected, was measured quantitatively. The weight of each rat was determined initially and then subsequently at intervals of one week up to mating. Animals of all subsequent generations were weighed at birth, 4, 12, and 21 days and subsequently at weekly intervals up to the time of mating. Pregnant dams were weighed on days 0, 7, 14, and 20 of pregnancy" (pp. 3-4).

According to the study authors, other parameters evaluated were: 1) pregnancey rate (fertility), 2) mating performance, gestation period, litter data (including litter size, litter weight and mean pup weight, 3) pup mortality [Note: in defining pup mortality, the study authors write: "Group A and mean B was (were?) calculated from individual litter values" p. 5. Which means?] 4) abnormalities [Comment: with respect to abnormalities the study authors write: "Where indicated, young were preserved for further examination of suspect organs" p. 5. How were suspect organs reported and characterized for our evaluation?] The study authors also say that "pups selected for rearing were chosen from as many litters and as close to the mean weaning weight as possible." [Question - what assurance do we have that this selection process did not exclude pups with abnormalities arising as a consequence of treatment?]

The teratologic phase of the study was conducted on selected rats. "On day 20 the 5 females in each group which were selected for teratological examination were killed by cervical dislocation and the ovaries and uterine contents examined immediately to determine:

a. The number of corpora lutea.
b. The number of viable young.
c. The number of resporption sites.
d. The litter weight, from which the mean pup weight is calculated.
e. Fetal abnormalities. All pups were examined on removal of uterus.

"One-third are preserved in Bouin's solution for subsequent free-hand sectioning to determine visceral abnormalities. The

remaining two-thirds were preserved in alcohol for subsequent dissection and examination under magnification by clearing and staining of the skeleton with alizarin to detect skeletal abnormalities" (p. 5).

Results:

F_0 Generation:

There were no remarkable compound related effects observed in this generation with regard to the following parameters: food consumption, body weight, general behavioral, physical effects or mortality. As determined from analyses on 6 animals of each sex in the F_0 generation (p. 8) brain cholinesterase was significantly inhibited (18%) by the 180 ppm regimen, and RBC cholinesterase was inhibited 30 and 37 percent, respectively by the 30 and 180 ppm diets, whereas in females brain cholinesterase was inhibited 23% at 180 ppm but plasma cholinesterase was inhibited 46 and 62 percent, respectively at 30 and 180 ppm. [Note: table 8 (p. 33) contains mean cholinesterase data. Individual cholinesterase data from all groups is found in volume 5, table 3 through 7, p. 01009. This table contains an inaccuracy with respect to the designations of various columns that identify the animal in question. Thus columns 2, 3, and 4 are designated in the legend as denoting generation, sex and dose group, respectively. However, the actual columns are not numbered but instead are marked S, D, and G, in this order, which presumably represent Sex, Date, and Generation, respectively, and hence, are ordered differently. Thus, if there were a master list of identification numbers for the individual animals, one could determine which set of column designations in this table is correct. However, no such master list has been located. The animals are identified in other tables, e.g., body weight, food consumption, etc., but are these more reliable? The study authors must correctly and unambiguously identify animals in table 3 to 7 for our inspection of cholinesterase data.]

F_1A and F_1B Generations:

Pregnancy rate is defined in this study as number litters born/number of females mated (p. 4). Table 4 indicates for F_1A very nearly 100 percent pregnancy rates for the four groups of animals. However, for the F_1B generation, pregnancy rates drop to 56 percent, 56 percent, 71 percent, and 72 percent, respectively, for the control, 3 ppm, 30 ppm, and 180 ppm groups. In attempting to explain this decline the following notation is introduced below table 4: "Pregnancy rate for the second mating in all cases lower as animals taken for teratology and pseudo-pregnant animals included in mated but not delivered animals." Our response to this is that we would like to see the actual number of pseudo-pregnant F_0 animals, i.e., how many F_0 dams in the second mating were in fact not pregnant. Apparently

there were essentially none in the first mating. Furthermore, if 5 pregnant dams/dose group were removed for teratology examination, the upper or limiting pregnancy rate for the remaining 20 dams/dose group, would be 80 percent. Comparing, for example, the 56 percent pregnancy rate with an 80 percent rate in the cases of the control and 3 ppm groups of the F_1B suggests a large number of pseudo-pregnancies occurred. This suggests a breeding problem in the second mating of F_0 animals.

Gestation periods for both matings were unaffected by NTN 8629, with the exception of a small but statistically significant shortening of the gestation period in the F_1B generation at the 180 ppm dose level (i.e., 22.1 vs 21.6 days).

Compound effects on litter weight and mean pup weight were unremarkable.

The study authors advise that pup mortality was unaltered except among F_1B pups at the 180 ppm dose level. No data are cited in support of this finding. This constitutes a deficiency. Nonetheless, a significant increase in mortality among F_1B pups in the 180 ppm group was reported. We must see the mortality figures in other dose groups.

With regard to teratology, 5 pregnant (20 days) females (F_0 of the second breeding which would yield the F_1B generation) were sacrificed to determine number of corpora lutea, resorption sites, viable young and weight of viable young. The results appear to be reported as the F_1B generation in table 9 (p. 34). We would be more inclined to refer to these as F_0 results since the pregnant animals did not actually deliver, but will accept the F_1B designation. According to the mean data presented for this generation in table 9 there were no remarkable effects on any of the parameters identified above. However, 5 pregnant animals is an insufficient number for any conclusion.

The study authors advise that there were no significant increases in skeletal or visceral anomalies in offspring of F_0 dams related to NTN 8629. Also there were no gross deviations observed at necropsy in the F_0 generation, according to study authors. Among the F_1 male study groups, brain, erythrocyte, and plasma cholinesterase were inhibited in the 180 ppm dose group only. Among F_1 females, the three cholinesterases were also inhibited in the 180 ppm group, but in addition erythroycyte cholinesterase was slightly inhibited in the 30 ppm dose group.

F_2B Generations:

During 12 weeks of observation there were no remarkable effects of NTN 8629 dosing on food consumption (table 1, p. 18), or on body weight of either sex of the F_1B generation. There were scattered changes in body weight, particularly among females, but we view these as of no toxicological significance.

Premating mortality was low and unremarkable.

Pregnancy rates for the two F_1B matings are presented in table 10 (p. 35). The pregnancy rate for the F_2A generation was 68 percent in the 180 ppm group as compared to 96 percent for the control. This is considered to be evidence of an adverse effect. In the F_2B generation the same comments offered above for pregnancy rate of the F_1B generation applies, i.e., rates in all dose groups are remarkably reduced presumably due to pseudo-pregnancies. We need actual tabulations on the number of pseudo-pregnant animals. Particularly surprising is the low pregnancy rate (32 percent) in the control group. This indicates a husbandry problem.

There were no remarkable compound related effects evident in the F_2A or F_2B generation with respect to average gestation period (table 5, p. 28) or litter size observations (table 6, p. 29) including such parameters as weight at birth, number of live pups at birth, number of females at birth, number of stillborns, etc.

Mean pup weight variations in all dose groups of F_2A and F_2B generations (table 7, p. 32) appear as only normal insignificant variations.

There were no effects of NTN 8629 on pup mortality.

Cholinesterase data obtained on the F_1B generation animals (table 8, p. 33) demonstrate significant inhibition of plasma, RCB and brain cholinestrases (25, 28 and 18%, respectively) in the 180 ppm males. This was also true among females, at this dose (corresponding inhibitions - 40, 33, and 23%). There was an additional significant finding of 18% inhibition in RBC activity of the 30 ppm dose group.

Teratology data (table 9, p. 34) for the F_2B generation show no compound-related effects on number of corpora lutea, number of resorption sites (though quite variable), number of viable young or weight of viable young.

F_2 Generation:

The F_2 generation growth and development factors of food consumption (table 1, p. 19), and body weight [tables 2C (p. 23) and 3 (p. 25)] were normal. We note that data in table 2C was attributed to the F_2 generation, we are unable to determine if it represents averages of F_2A and F_2B data? The table should also include n (number of observations) for the various mean values. Also we note in table 2C that weight gain in all dose groups over the 12-week observation period is less for females, this being true among controls and all dosed groups. Generally speaking male rats achieved in 3 weeks a weight range approximating

240 grams, a weight range achieved in females only after 12 weeks. We can suggest no explanation for this save poor husbandry. (This observation of male vs female mean body weight data is apparent in F_0, F_1, and F_3 generations also.)

No data on daily observations of the F_2 generation were presented for examination.

Cholinesterase data on the F_2 (presumably F_2B) generation (table 8, p. 33) shows that brain cholinesterase was not inhibited in animals of either sex at any dose level. The RBC enzyme was inhibited 52% in males at the 180 ppm dose, and in females the RBC and plasma enzymes was inhibited 27 and 24 percent, respectively, in the 180 ppm group. No other significant inhibitions were observed at any dose.

Pregnancy rates for the three matings of the F_2B generation to produce the F_3A, F_3B, and F_3C generations are presented in table 11 (p. 36). These data share the features characteristic of previous matings in that matings subsequent to the first yielded reduced pregnancy rates. The same explanation is offered, namely pseudo-pregnancies. As before, the actual data on number of pseudo-pregnancies should be submitted. The pregnancy rate appears reduced in the 180 ppm group compared to the control in F_3C (29% vs 43% pregnancy rate, respectively).

Table 5 shows that the average gestation period during the F_3A and F_3B pregnancies was not altered by NTN 8629. For an unknown reason gestation period was not reported for F_3C generation, which suggests a problem.

With regard to litter size, the data reported in table 6 (p. 29) indicate that litter size was down in the F_3B and F_3C generations in the control as well as in various dose groups. This is indicative of poor husbandry. The study authors indicate that, "When compared to controls, litter size was not adversely affected by ingestion of NTN 8629" (p. 11). While this may be strictly true with respect to litter size, table 6 (pp. 30 to 31) shows that at the 30 ppm NTN 8629 dose level in the F_3C generation, the number of live pups at weaning and the weight of live pups at weaning were markedly reduced with respect to the control values, the former being statistically significant. Also, data on the 180 ppm dose group of F_3C are not reported. These findings indicate a negative influence of NTN 8629 at 30 ppm on reproduction. [Note: since effects on the spermatogonium were observed in the first multigeneration study conducted at Albany, it is unacceptable that this phenomenon was not specifically evaluated and commented upon in this second study.] This adverse effect in the third generation is reminiscent of the findings in the first multigeneration study.

According to the study authors there was no pup mortality related to NTN 8629 in the diet; however, the petitioners should be advised to submit the data which will substantiate this claim.

With regard to cholinesterase assays, table 8 (p. 33) simply reports mean data for F_3 males and females. Since the protocol is ambiguous as previously described, we cannot determine which F_3 generation litter was evaluated. Nonetheless, data in table 8 show no significant inhibition of brain cholinesterase, but do show slight inhibition at 30 ppm but not 180 ppm, for plasma cholinesterase in females.

In the F_3B generation, teratologic examination of selected pregnant F_2B dams revealed no significant findings except reductions in the number of corpora lutea in the 30 ppm and 180 ppm dose groups.

F3 Generation

With regard to F_3 generation pups (and we are not sure which of the three F_3 generation litters is referred to) no effects of NTN 8629 were observed on food consumption. In table 2d (p. 24), if stars indicate statistical significance, it is to be concluded that NTN 8629 exposure induced increases in body weight gain (males only) at all dose levels. The NOEL for this effect is indeterminate from data provided, but is somewhere below 3 ppm. An explanation for this effect has not been established.

There were no gross or microscopic abnormalities observed in the F_3B generation. Hence, there is not a finding of increased developmental abnormalities or teratogenesis.

Excess pneumonia was observed among males of the high-dose groups. The explanation for this is not clear.

Summary Remarks

° In consideration of the entire report, there is ambiguity and considerable confusion in the study as written and tabulated. Plasma cholinsterase NOEL = 30 ppm for both sexes.

° Cholinsterase inhibition as characterized by no-effect levels is summarized in tabular form as follows:

Generation	Cholinesterase NOEL, ppm					
	Plasma		Erythrocyte		Brain	
	M	F	M	F	M	F
F_1	30	30	30	3	30	30
F_2	180	30	30	30	180	180
F_3	180	3	30	30	180	180

In general it is to be noted that the magnitude of changes in cholinesterase levels as disclosed in this study are not great, and are to be questioned as indicating truly significant inhibitions of the enzymes.

° Declining pregnancy rates were reported in breedings subsequent to the first of the F_0, F_1B, and F_2B generations, this is evidence of poor husbandry.

° A significant increase in pup (F_1B) mortality was reported in the 180 ppm dose group.

° A small decrease in gestation period was reported for F_0 dams pregnant with the F_1B generation in the 180 ppm dose group.

° The pregnancy rate for F_1B dams in the first breeding was 68 percent in the 180 ppm group as compared to 96 percent for the control group. This is evidence of an adverse effect of NTN 8629 on pregnancy.

° Gestation periods were not reported for F_2B dams pregnant with the F_3C generation. Apparently, there was a problem with this generation.

° The number of live pups in the F_3C generation, 30 ppm dose group, was reduced with respect to controls, i.e., 1.6 ± 1.7 vs 5.0 ± 1.0. For reasons not explained by the study authors, no data are provided on the number of live pups in the 180 ppm dose groups. Such evidence is indicative of a negative influence of 30 ppm NTN 8629 on reproduction.

° Furthermore, there were significant increases in hydronephrosis among the F_3B fetuses of the F_2B dams in dose groups 30 and 180 ppm (p. 12). The NOEL = 3 ppm for this finding of compound-related developmental toxicity.

° In the F_3 generation significant increases in the body weight of male rats were observed in all dose groups at many time points during the 12-week observation period. This evidence suggests an NTN 8629 effect even at 3 ppm in the diet.

° In the first multigeneration study on NTN 8629, dated February 1, 1978 spermatogonial karyopyknosis was observed in all middle (50 ppm, diet) and high (500 ppm, diet) dose group rats examined. This finding was inadequately pursued in the earlier study, and not mentioned in this current study. Why not?

° The petitioner should clarify all ambiguities and make certain all relevant Japanese is interpreted properly.

° Histopathology reports are provided (vol. 5, tab J) for F_3 generation animals (111 days postpartum) covering approximately 25 animals/sex/dose. Whether F_3A, F_3B, of F_3C is the group referred to remains uncertain. There were no findings that could be linked to dosing, except perhaps lung effects (i.e., pneumonia) in males at high doses. The fact that hydronephrosis was not observed in offspring of this age (i.e., 111 days) would suggest that hydronephrosis observed prenatally is reversible and should be consider an aspect of developmental toxicity as indicated above rather than teratogenicity.

° Core Classification: Supplementary

General Comments Concerning the Various
Tables Listed by Letter Under Tab No. 17 of
Volume 5 for this Multigeneration Study.

Tab A - Food consumption data - top line cannot be read (presumably represents weeks). Does appear useful. Some pages have extra numbers toward the margin which I do not readily understand.

Tab B - Contains gross observations at necropsy (table 2-11). This table appears without introduction or adequate explanation. The legend indicates "All organs normal unless otherwise noted" (p. 1). Does this mean that all animals in the various groups were examined (how many would this be?) and found normal excepting the abnormalities reported here. Presumably, using the animal number columns, one could determine exactly which group the animal came from. Is it incumbent upon the reviewer to perform this task? One would like to see a definitive statement such as: 25 males, 25 females in groups F_0, F_1B, F_2B, and F_3B (or F_3C) at each dose level were examined grossly. Those which exhibited abnormalities are so recorded here, all others were normal. But there is no such statement. One doesn't know with certainty how many animals from the various groups were examined. Nonetheless, in scanning these tables we note the somewhat frequent appearance of hemorrhagic conditions in various organs which always appear in the high dose groups.

Tab C (Table 3-1)

Body weight data. A problem with this and other tables is that generations are defined simply as 1, 2, 3, 4, = F_0, F_1, F_2, and F_3, respectively. We don't know for instance whether F_2 refers to F_2A or F_2B or both; perhaps this could be determined using the rat number from a master table somewhere which identified rats according to number, but such a master table has not been located.

Tab D - Body weight data for pregnant dams.
This appears to be a reasonably good table for identifying dam weights of individual animals at 0, 7, 14, and 20 days of gestation.

Tab E - This table has no identification or legend and is not readily apprehended.

Tab F - Body weight data for offspring.
First page is out of order. However, this does appear to be a satisfactory table for determining individual pup weights for time points at birth, 4, 12, and 21 days of age (weaning).

Tab G - Table 3-5
Contains gestation lengths for the various matings. Appears to be a reasonable table for getting individual gestation lengths. After 1st and 2nd matings, but apparently not for the third F_2 mating.

Tab H - Table 3-7

Cholinesterase data.
Columns are not unambiguously identified. Legend indicates the order to be that of generation, sex, dose. However, column headings show the order, S, D, G.

Tab I

A. Data file of frequency counts of anonomalies. This table is not readily apprehendable.

B. "Table 3 to 8 continued." Comment: continued from where? Appears to be examinations (external and internal) of 84 fetuses. They come from 5 dams in each dose group of the F_1B generation.

Hemorrhages appear often but not in a dose dependent manner. According to the authors "The hemorrhages in the abdominal cavity, plural cavity under the skin in the skeletal muscles including extremities cannot be explained at this time. They are definitely not dose-related" (p. 14). We concur with this opinion expressed by the authors with respect to this table. There were no other remarkable observations.

Similarly in the F_2 generation hemorrhages were often noted among the fetuses of the five dams sacrificed in each group; but this was not a dose-related phenomenon, in fact it seems more prevalent in controls. There were no other remarkable teratogenic findings.

Tab J -
Includes no adequate legend. Contains histopath sheets for F_3 generation animals, males and then females, covering approximately 25 animals/sex/dose. There is uncertainty as to whether these are F_3A, F_3B, or F_3C, i.e., still needed is a master I.D. sheet.

Having reviewed all of these histopath sheets there is nothing that could be linked to dosing except perhaps lung effects in males at high doses (i.e., pneumonia, etc.) which we do not consider to be compound related, except perhaps in an indirect way, such as weakened condition of animals.

Study 1: Mutagenicity Test on Bacterial Systems with Prothiophos Nitokuno Agricultural Chemical Institute, Laboratory of Toxicology, Report No. 38, January 24, 1977 (Authors: H. Inuaki and A. Iyatomi)

(1) Rec-Assay

DNA-damaging capacity of Prothiophos was investigated by the Rec-Assay using two strains of Bacillus subtilis, NIG45 (Rec^-, repair deficient strain) and NIG17 (Rec^+, wild type strain), according to the method described by Kada et al. (Mutat. Res. 16:165-174, 1972). Both overnight cultures of these two strains of B. subtilis were streaked on the surface of solid agar broth. The test compound was dissolved in DMSO, and applied to a paper disc (10 mm in diameter) which was placed on the edges of culture streaks. The plates were incubated at 37 °C overnight, and then the diameter of growth inhibition zone (mm) was measured. Mitomycin C (positive control) was run concurrently with the test compound.

Results:

Treatment	Concentration ug/disc	Inhibition Length (mm) NIG17	Inhibition Length (mm) NIG45	Difference (mm)
Mitomycin C	3	1	9	8
Prothiophos	300	0	0	0

Findings:

The growth of both strains of B. subtilis was not inhibited by Prothiophos at the concentration tested. However, the growth inhibition between the NIG17 and NIG45 was significantly different by the positive control compound (Mitomycin C, 300 ug/disc). Therefore, Prothiophos (95% purity) gave negative results in the Rec-Assay.

Evaluation:

This study is judged inadequate and unacceptable. The following deficiencies in performing the DNA-repair assay with B. subtilis were noted:

1. Because of the poorly diffusable property of the test compound dissolved in DMSO, it did not yield interpretable results (e.g., no test results in the spot test. This test should be conducted using higher concentration of Prothiophos per

plate if possible. If the spot test assay is apparently unable to obtain interpretable results, the test compound should be further evaluated in the modified liquid suspension method described by Slater, Anderson, and Rosenkranz (Cancer Res. 31:970-973, 1970).

2. Bacteria should be exposed to the test compound both in the presence and absence of an appropriate metabolic activation in this study. This study with metabolic activation was missing in the report.

3. The described procedure for standardizing bacterial cultures in this study was inadequate. Both bacterial cell suspensions of the tester strains of B. subtilis (NIG17 and NIG45) should be standardized to equally desired density of viable cells prior to testing. The phase of bacterial growth should be documented. In evaluating the differential inhibition of these two bacterial strains, the sensitivity of the assay is largely dependent on the number of organisms seeded in the agar overlay.

(2) Reversion Assay With In Vitro Metabolic Activation

The mutagenicity of Prothiophos dissolved in DMSO at 3 concentrations (0.1, 10, and 1000 ug/plate under activation; 1000 ug/plate under nonactivation) was evaluated by the plate incorporation procedure described by Ames et al. (Mutat. Res. 31:347-364, 1975). Four histidine-requiring strains of Salmonella typhimurium (TA98, TA100, TA1535, and TA1537) were used in the Salmonella reversion assay. The in vitro mammalian metabolic activation system consisted of liver homogenate "S-9" either from the phenobarbital induced rats or mice and also cofactor solution described by Ames. The test compound was tested in the Ames Salmonella reversion assay with metabolic activation either by the rat liver microsome or the mouse liver microsome separately. Positive controls and solvent control were also run concurrently with the test compound.

Results:

Number of His^+ Revertant Colonies Per Plate

Treatment	Conc. (Per Plate)	TA1535 -S9	TA1535 +S9	TA1537 -S9	TA1537 +S9	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9
1st Trial - Rat Liver									
DMSO	--	--	4	--	15	--	12	--	248
AAF	50 ug	--	--	--	--	--	1000*	--	--
AF-2	0.05 ug	--	--	--	--	1000*	--	1000*	--
NTG	10 ug	1000*	--	--	--	--	--	--	--
Dexon	30 ug	--	--	328*	--	--	--	--	--
Prothiophos	0.1 ug	--	3	--	10	--	9	--	262
"	10 ug	--	7	--	17	--	19	--	242
"	1000	2	2	16	14	6	9	330	234
2nd Trial - Mouse Liver									
DMSO	--	--	4	--	6	--	29	--	198
DMNA	50 ug	--	1000*	--	--	--	--	--	1000*
AF-2	0.05 ug	--	--	--	--	1000*	--	1000*	--
NTG	10 ug	1000*	--	--	--	--	--	1000*	--
Dexon	30 ug	--	--	376*	--	--	--	--	--
Prothiophos	0.1 ug	--	7	--	21	--	13	--	208
"	10 ug	--	17	--	16	--	27	--	218
"	1000	4	3	16	16	30	22	296	276

Postive Controls: AF-2 = Furylfuramide; NTG=N-methyl-N-Nitro-N-Nitrosoguanidine; DMNA = Dimethylnitrosamine. *Positive response.

Findings:

The test compound, Prothiophos, was not mutagenic in the Ames Salmonella reversion assay with the metabolic activation by the rat or mouse liver microsomes at the concentrations tested.

Evaluation:

This appears to be an inadequate study, not conducted according to the accepted procedures of the Ames Salmonella/Mammalian Microsomal Mutagenicity Test. The study is judged inconclusive and unacceptable in the present form. The following inadequacies in performing the Ames Test were noted:

1. Since the number of spontaneous revertant colonies from TA 100 strain (e.g., 198 to 248 revertant colonies/plate) was not demonstrated in the acceptable, normal range (120 to 200 revertant colonies/plate) recommended by the Ames Test (Mutat. Res. 31:347 to 364, 1975), it is questionable that the integrity of the culture was properly maintained in performing the assay.

2. The specific procedure for confirming the genotypes of tester strains was not presented with the report.

3. Bacteria should be exposed to the test compound both in the presence and absence of metabolic activation in this study. The complete results of this study without metabolic activation at the selected concentrations of Prothiophos were missing in the report.

4. The selected exposure concentrations of the test compound should be based on the preliminary toxicity range-finding study. Toxicity may be evidenced by a reduction in the number of spontaneous revertants, a clearing of the background lawn or by the degree of survival of treated cultures. The rationale for selection of dose in this study was not given.

5. The complete results from the positive mutagenesis controls (AAF for the 1st trial and DMNA for the 2nd trial) to ensure the efficacy of the activation systems (rat and mouse liver microsomes) were lacking in this study (e.g., no data were given for TA1535, TA1537, and TA100 strains in 1st trial and also no expected response for TA1537 and TA98 strains in 2nd trial).

Study 2: Mutagenicity Test on Bacterial Systems with Prothiopos Nitokuno Agricultural Chemical Institute, Laboratory of Toxicology, September 25, 1978 (Authors: Y. Shirasu, M. Moriya, and T. Miyazawa)

(1) Rec-Assay

DNA-damaging capacity of Prothiophos (94.7% purity) was investigated by the Rec-Assay using two strains of Bacillus subtilis, M45 (Rec^-, repair deficient strain) and H17 (Rec^+, wild type strain) according to the method described by Kada (Mutation assay of chemical substances Kodansha 31 to 44, 1973) and Shirasu et al., (Mutat. Res. 40:19 to 30, 1976). Both test strains were streaked on B-II agar plate medium. The test compound was dissolved in DMSO and applied to paper disc (10 mm in diatmeter) which was placed on the agar plate surface to cover the starting point of the streak. The plates were incubated for 24 hours at 37 °C and then the diameter of growth inhibition zone was measured. Kenamycin (negative control) and Mitomycin C (positive control) were run concurrently with the test compound.

Results:

Treatment	Concentration ug/disc	Inhibition Length (mm) M45	Inhibition Length (mm) H17	Difference (mm)
DMSO	0	0	0	0
Kenamycin	10 ug/disc	4	3	1
Mitomycin C	0.1 ug/disc	9	1	8
Prothiophos				
"	1%	0	0	0
"	5%	0	0	0
"	10%	0	0	0
"	25%	0	0	0
"	50%	0	0	0
"	300%	0	0	0

Findings:

The growth of H17 and M45 strains of Bacillus subtilis was not inhibited by Prothiophos at the concentrations tested. However, the growth inhibition between the M45 and H17 was significantly different by the positive control compound (Mitomycin C, 0.1 ug/disc). Therefore, Prothiophos was stated as negative in the Rec-Assay.

Evaluation:

The results of this study appear inadequate to support the conclusion drawn in the report. The critical deficiencies in performing this test have been pointed out in the previous Rec-Assay study (Nitokuno Agricultural Chemical Institute, Laboratory of Toxicology Report No. 38, January 24, 1977). Therefore, the study is judged inconclusive and unacceptable.

(2) Reversion Assay

The mutagenicity of Prothiophos (94.7% purity) dissolved in DMSO at 6 concentrations (10, 50, 100, 500, 1000, and 5000 ug/plate) was evaluated by the plate incorporation assay described by Ames et al., (Mutat. Res. 31:347 to 364, 1975). Five histidine-requiring strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537, and TA1538) and one tryptophan-requiring strain of E. coli (WP2 her) were used in this reversion assay. The in vitro mammalian metabolic activation system consisted of liver homgenate "S-9" from Arocolor induced rats and also cofactor solution described by Ames. Positive controls and solvent control were also run concurrently with the test compound.

Results:

Mean Number of His^+ Revertant Colonies Per Plate

Treatment ug/plate	WP2 her		TA1535		TA100		TA1537		TA1538		TA98	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
DMSO	17	11	4	4	120	123	6	7	17	18	17	21
2-AA 10 ug	--	187*	--	202*	--	3000*	--	608*	--	3000*	--	3000*
AF-2 .05 ug	--	--	--	--	1119*	--	--	--	--	--	--	--
" 0.1 ug	--	--	--	--	--	--	--	--	--	--	312*	--
" .25 ug	2000*	--	--	--	--	--	--	--	--	--	--	--
9-AA 200 ug	--	--	--	--	--	--	10000*	--	--	--	--	--
2-NF 50 ug	--	--	--	--	--	--	--	--	3000*	--	--	--
3-PPL 30 ug	--	--	828*	--	--	--	--	--	--	--	--	--
Prothiophos												
10 ug	15	14	10	6	110	124	4	4	14	18	17	25
50 ug	12	15	7	4	91	112	8	7	13	12	17	22
100 ug	13	17	4	2	101	121	4	8	9	17	16	28
300 ug	15	15	5	3	105	136	6	7	9	11	24	19
1000 ug	13	20	5	7	97	123	3	6	14	13	18	19
3000 ug	16	15	3	9	94	123	4	6	8	13	21	19

Postive Controls: 2-AA = 2-Aminoanthracene; AF-2 = Furylfuramide; 9-AA = 9-Aminoacridine; 2-NF = 2-Nitrofluorene; 3-PPL = 3-propiolactone. *Positive response.

Findings:

Results of this study showed that no evidence of mutagenic activity was observed at any concentration of the test compound tested in the presence and absence of metabolic activation. The strain specific control compounds (e.g., AF-2, 9-AA, 2-NF, and 3-PPL) and the positive control compound (2-AA) to ensure the efficacy of the activation system have given the positive responses as expected.

Evaluation:

Under the test conditions reported, the following deficiencies in this study are observed:

1. The specific procedure for confirming the genotypes of tester strains of Salmonella typhimurium were not presented with the report.

2. The selected exposure concentrations of the test compound should be based on the preliminary toxicity range-finding study. Toxicity may be evidenced by a reduction in the number of spontaneous revertants, a clearing of the background lawn or by the degree of survival of treated cultures. The rationale for selection of dose in this study was not given.

3. The bacterial cell suspension of each tester strain at the late exponential phase of growth must be standardized prior to testing. The bacteriological procedures used for standardizing the bacterial cell suspension to a desired density of viable cells per milliliter were not included in this report.

4. The described procedures for the preparation of medium were inadequate. The source of nutrient broth for growing the bacterial cultures and the composition of mutant selective medium must be identified in the report.

The study may be upgraded to be acceptable on resolution of the reporting deficiencies.

Study: Tokuthion. Antidotal Test on Rats and Mice.

Laboratory: Nitokuno, Agricultural Chemicals Institute, Laboratory of Toxicology

Study Number and Date: Tab 29; June 1, 1977

Accession Number: 073098

Material Tested: NTN 8629 (95% purity)

Animals: Wistar male rats (110 to 130 gm body weight); dd male mice (23 to 25 gm body weight)

The purpose of this study was to determine the effectiveness of atropine, 2-PAM and toxogonine (BH6) and reduced glutathione (GSH) as antidotes for the acute toxic effects of NTN 8629 induced in the rat and mouse.

Methods and Materials:

For these studies technical grade tokuthion (NTN 8629) of 95 percent purity, sample dated March 1975, was employed. Animals used in the study were Wistar male rats (110 to 130 gm body weight) and dd male mice (23 to 25 gm body weight).

The study procedure is presented as follows:

> "This compound was dissolved in olive oil at given concentrations, and orally applied to receive 0.5 ml/100 g for rats and 0.1 ml/10 g for mice. Further, the following reagents were prepared as antidotes: atropine-sulfate 2-PAM, Toxogonine (BH6) and reduced glutathion (GSH). These antidotes were dissolved in the physiological saline, and were intraperitoneally injected in volume of 0.2 ml/100 g for rats and mice (p. 2)."

In all studies the post-treatment observation period was 7 days.

In a study employing 10 mice per dose group, animals were administered single oral doses of tokuthion at the rate of 1000 mg/kg with and without antidote.

Results:

Mouse mortality observed in the various groups 7 days following administration of tokuthion is summarized in the following table (p. 4).

Antidote	Dose mg/kg	Time, hrs	Mortality (7 day)
None			9/10
Atropine sulfate	50	3	4/10
Atropine sulfate	25 x 3	3, 12, 24	3/10
2-PAM	50	3	7/10
2-PAM	25 x 3	3, 12, 24	4/10
BH6	25	3	5/10
BH6	10 x 3	3, 12, 24	5/10
GSH	50	3	8/10
GSH	25 x 3	3, 12, 24	7/20

In both the mouse and rat studies, additional mortality data are provided for days 1, 2, 3, and 5 following administration of tokuthion. These data provide mortality trends which support the following conclusions based upon the 7-day mortality data.

Conclusions:

In both species atropine sulphate at the dose employed provided antidotal effects, particularly when administered in multiple doses, consistent with the total quantity of atropine administered. 2-PAM appeared to be more effective in the mouse than in the rat. BH6 appeared to be considerably more effective in the mouse than in the rat. GSH was not remarkably effective in either species.

Based upon results in the preceding studies in the rat and mouse, dosages were selected for the following more comprehensive study. Accordingly, male rats and male mice in groups of 10 each were administered tokuthion over a range of dosages (rats: 1000, 1500, 2000, and 3000 mg/kg; mice: 500, 1000, 1500, and 2000 mg/kg) to determine the time course of mortality and LD_{50} values in both the absence and presence of the various antidotes and combinations of antidotes.

Results:

The following table (modified form of that in the text, p. 5) indicates dosage and frequency of administration of antidotes and resulting LD_{50} values for both animal species.

Antidote treatment (i.p.)	rats - 4 hrs mice - 3 hrs	24 hrs 12 hrs	48 hrs 24 hrs	LD_{50}, (mg Tokuthio kg) Rats	Mice
1. No treatment	--	--	--	1200	900
2. Atropine	50 mg/kg	50 mg/kg	20 mg/kg	1550	1450
3. 2-PAM	50 mg/kg	50 mg/kg	20 mg/kg	1400	1150
4. BH6	25 mg/kg	25 mg/kg	10 mg/kg	1300	1000
5. GSH	50 mg/kg	50 mg/kg	20 mg/kg	1200	1000
6. Atropine + 2-PAM	50 + 50 mg/kg	50 + 50 mg/kg	20 + 20 mg/kg	1550	1550
7. Atropine + BH6	50 + 25 mg/kg	50 + 25 mg/kg	20 + 20 mg/kg	1400	1300
8. Atropine + GSH	50 + 50 mg/kg	50 + 50 mg/kg	20 + 20 mg/kg	1500	1350

Results of this study indicate that at dosages employed, atropine is the most effective antidote of those studies. 2-PAM also exhibited antidotal activity, but when administered in combination with atropine, there was no enhancement in the effectiveness of atropine alone in rats, and only a marginal enhancement of that seen in mice. BH6 when administered alone demonstrated weak antidotal activity. GSH alone was virtually ineffective. BH6 and GSH in combination with atropine were not as effective as atropine alone.

Conclusions:

1. LD_{50} values for tokuthion in male rats and mice are 1200 and 900 mg/kg, respectively.

2. At dosages employed, atropine exhibited appreciable activity as an antidote for tokuthion.

3. 2-PAM exhibited some antidotal activity, but was less effective than atropine.

4. 2-PAM and atropine administered together were no more effective than atropine alone.

5. BH6 and GSH were essentialy noneffective as antidotes.

Core Rating:

Supplementary

Study: Study to Evaluate Effect of Antidotes After Oral Adminstration of NTN 8629.

Laboratory: Bayer AG, Institute Für Toxikologie, Wuppertal-Elberfeld

Study Number and Date: Volume 2, Tab 28; November 10, 1975

Accession Number: 073093

Material Tested: NTN 8629 (93.2% purity)
Batch 1604/74 dated April 1974

Animals: Wistar II Female Albino Rats.

The purpose of this study was to determine the effectiveness of atropine, 2-PAM and toxogonin (obidoxime chloride) as antidotes for the toxicity induced in the rat by NTN 8629.

Methods and Materials:

Animals employed in this study were Wistar II albino rats weighing 175 to 190 grams. The animals were fed Altromin standard diet and water ad libitum. The experimental procedure is described as follows:

> "Groups of 15 female rats received a single oral dose of NTN 8629 emulsified in a mixture of Cremophor and water. Four to 23 hours after NTN 8629 administration, shortly before onset of the poisoning symptoms, the rats received intraperitoneal injections of the following antidotes and antidote combinations, formulated in physiological saline solution: 50 mg atropine sulphate/kg body weight, 50 mg PAM/kg, 20 mg Toxogonin/kg, 50 mg atropine sulphate + 50 mg PAM/kg, and 50 mg atropine sulphate + 50 mg Toxogonin/kg. A control group was treated only with NTN 8629 and received no antidotes. After the application, the rats were kept under observation for 14 days" (p. 5).

When dosages of NTN 8629 ranging from 750 to 1500 mg/kg were employed, the following results were obtained. Without antidote, all dosages of NTN 8629 employed produced toxic symptoms in all animals of every dose group. At 750 mg/kg, one rat in 15 died, at 1000 mg/kg, 11 of 15 died and at 1500 mg/kg, all 15 rats of the group died. The LD_{50} for the control group was 945 mg/kg. When atropine sulphate was administered as described, all animals in all dose groups still exhibited toxic symptoms. However, none died at the low dose of NTN 8629 (750 mg/kg), 4 of 15 died in the 1000 mg/kg dose group and 15 of 15 died in the 1500 mg/kg dose group. The LD_{50} was 1095 mg/kg. With 2-PAM alone, a little more protection was seen than with atropine alone as antidote. The LD_{50} with 2-PAM alone was increased to 1108 mg/kg. Toxogonin alone similarly

increased the LD_{50} to 1012 mg/kg. Atropine sulphate plus 2-PAM as antidotes provided no benefit beyond that of either alone, the LD_{50} being 1038 mg/kg. An additional antidotal effect was seen with atropine and toxogonin together than with either alone. The LD_{50} for the combination was 1172 mg/kg.

Conclusion:

All three antidotes alone and those tested in combination exerted a protective or antidotal effect against NTN 8629 induced acute toxicity. Atropine sulphate in combination with toxogonin was most effective.

Core Rating:

Supplementary

13544

011864

Chemical:	**Prothiophos**
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